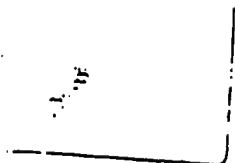


1 agggagagggc agtgaccatg aaggctgtgc tgcctgccc gtgagggca  
 51 ggctggccc tgcagccagg cactgcccgt ctgtgctact cctgcaaagc  
 101 ccaggtgagc aacgaggact gccctgcaggt ggagaactgc acccagctgg  
 151 gggagcagtg ctggaccgcg cgcaiccgcg cagttggcct cctgaccgtc  
 201 atcagcaaag gcctgcagct gaactgcgtg gatgactcac aggactacia  
 251 cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgccca  
 301 gcgggggccca tgcctgcag ccggctgccc ccacccctgc gctgctccct  
 351 gcactcggcc tgcctgctctg gggaccggc cagctatagg ctctgggggg  
 401 ccccgctgca gccacactg ggtgtgtgtc ccaggccct tgtgccactc  
 451 ctacagaaac ctggcccagt gggagcctgt cctggctcct gaggcacatc  
 501 ctacgcgaag ttgaccatg tatgtttgca cccctttcc cnaaccctg  
 551 accttccat gggcctttc caggatccn accnggcaga tcagttag  
 601 tganacana cgcctgcag atggccctc caacnntn tgttgnnt  
 651 tccatggccc agcatttcc acccttaacc ctgtgtcag gcactttc  
 701 cccaggaag cctccctgc ccacccan tatgaattga gccaggttg  
 751 gtccgtgtgtg tccccgcac ccagcagggg acaggcactc aggagggccc  
 801 agt22ggct gagatg22gt ggactg22gt g22ctggagg ac22ag22gt  
 851 acgtg22gt c22gg22gt cc22g22gt g22ctggagg cctgg22gt  
 901 gggggccaggc ctacacatg tggggctccc g22tggcagc ctgagc22g  
 951 cgtaggccct t22t22cac ctgnggata agcc22222 2222222

FIGURE 1A

MXA V L L A L L M A G L A L Q P G T A L L C Y S C K A Q V S N E D C L Q V  
E N C T Q L G E Q C W T A R I R A V G L L T V I S K G C S L N C V D D S  
Q D Y Y V G K K N I T C C D T D L C N A S G A H A L Q P A A A I L A L L P A L  
G L L L W G P G Q L

FIGURE 1B



09855153-054401

1 ATGAAGACAGTTTTTTTTATCCTGCTGGCCACCTACTTAGCCCTGCATCCAGGTGCTGCT  
 TACTTCTGTCAAAAAAATAGGACGACCGGTGGATGAATCGGGACGTAGGTCCACGACGA 60  
 M K T V F F I L L A T Y L A L H P G A A  
 61 CTGCAGTGCTATTCATGCACAGCACAGATGAACAACAGAGACTGTCTGAATGTACAGAAC  
 GACGTCACGATAAGTACGTGTCGTGTCTACTTGTGTCTCTGACAGACTTACATGTCTTG 120  
 L Q C Y S C T A Q M N N R D C L N V Q N  
 121 TGCAGCCTGGACCAGCACAGTTGCTTTACATCGCGCATCCGGGCCATTGGACTCGTGACA  
 ACGTCGGACCTGGTCGTGTCAACGAAATGTAGCGCGTAGGCCCGGTAACTGAGCACTGT 180  
 C S L D Q H S C F T S R I R A I G L V T  
 181 GTTATCAGTAAGGGCTGCAGCTCACAGTGTGAGGATGACTCGGAGAACTACTATTGGGC  
 CAATAGTCATTCCCGACGTCGAGTGTCACTCTACTGAGCCTCTTGATGATAAACCCG 240  
 V I S K G C S S Q C E D D S E N Y Y L G  
 241 AAGAAGAACATCACGTGCTGCTACTCTGACCTGTGCAATGTCAACGGGGCCACACCCTG  
 TTCTTCTGTAGTGACGACGATGAGACTGGACACGTTACAGTTGCCCCGGGTGTGGGAC 300  
 K K N I T C C Y S D L C N V N G A H T L  
 301 AAGCCACCCACCACCTGGGGCTGCTGACCGTGTCTGACGCTGTTGCTGTGGGGCTCC  
 TTCGGTGGGTGGTGGGACCCGACGACTGGCACGAGACGTGGGACAACGACACCCCGAGG 360  
 K P P T T L G L L T V L C S L L L W G S  
 361 AGCCGTCTGTAGGCTCTGGGAGAGCCTACCATAGCCCGATTGTGAAGGGATGAGCTGCAC  
 TCGGCAGACATCCGAGACCCTCTCGGATGGTATCGGGCTAACACTTCCCTACTCGACGTG 420  
 S R L  
 421 TCCACCCACCCACACAGG  
 AGGTGGGTGGGGTGTGTCC 441

FIGURE 2

09855153-051401

1 M K I F L P V T T R A M L N G V E R A S S NSCA-2  
1 M K A V L L A L L M A G R A L O C G T A MPSCA  
1 M K T V L L L L L A T V T A L H P G A A mPSCA  
  
21 L M C F S C L N O K S N L Y C E K P T I  
21 L L C Y S C K A Q V S N E D C L O V E N  
21 L Q C Y S C T A Q M N N R D C L N V Q N  
  
41 C S D Q D N Y C V T V S A S X G I G N L  
41 C T O L G E O C W T A R I R A V G L L T  
41 C S L O Q H S C F T S R L R A I G L V T  
  
61 V T F G H S L S K T C S P A C P I P E G  
61 V - - - - I S K G C S L N C V D D S Q  
61 V - - - - I S K G C S S Q C E D D S E  
  
81 V N V G V A S M G I S C C O S F L C N F  
76 D Y Y V G K K - N L T C C O T D L C N A  
76 N Y Y L G K K - N L T C C Y S D L C N V  
  
101 S A X D G G L R A S V T T E G A G F F L  
95 S G A H A L O P A A A L F A L L P A E G  
95 N G A H T L X P P T T L G E E T V E C S  
  
121 S L L P A L L R F G P  
115 L L L W G P G O K - -  
115 L L L W G S S R L - -

FIGURE 3



09855153-051401

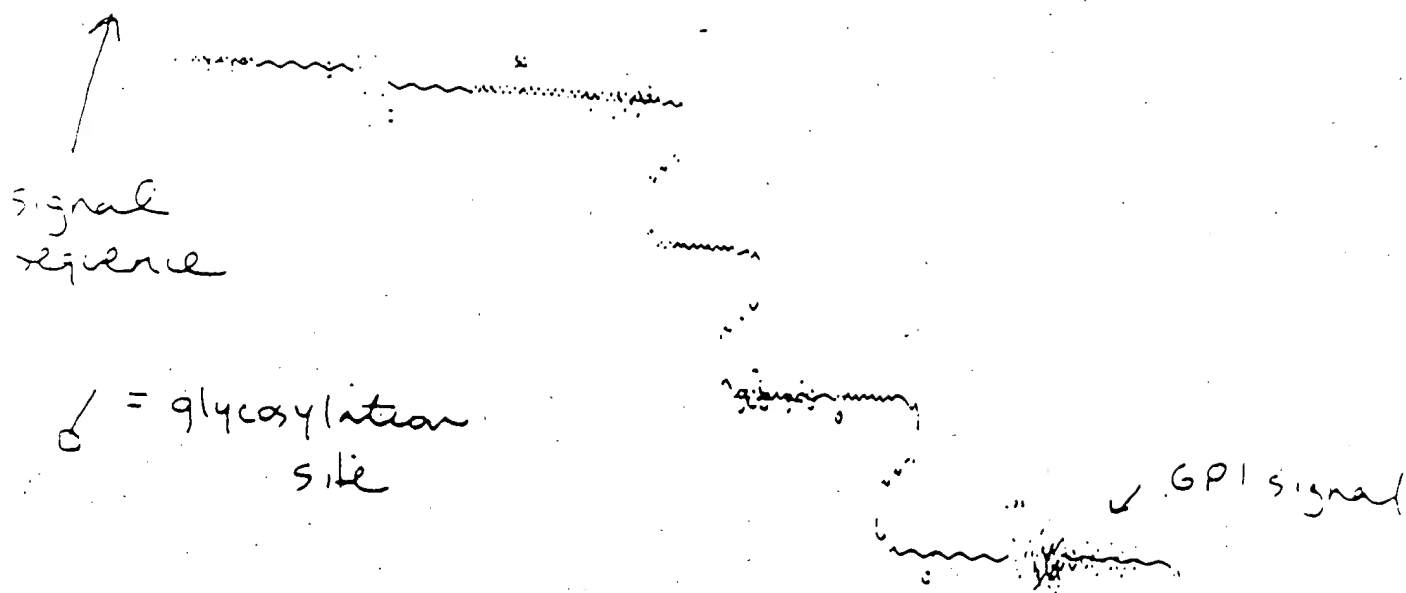


FIGURE 5

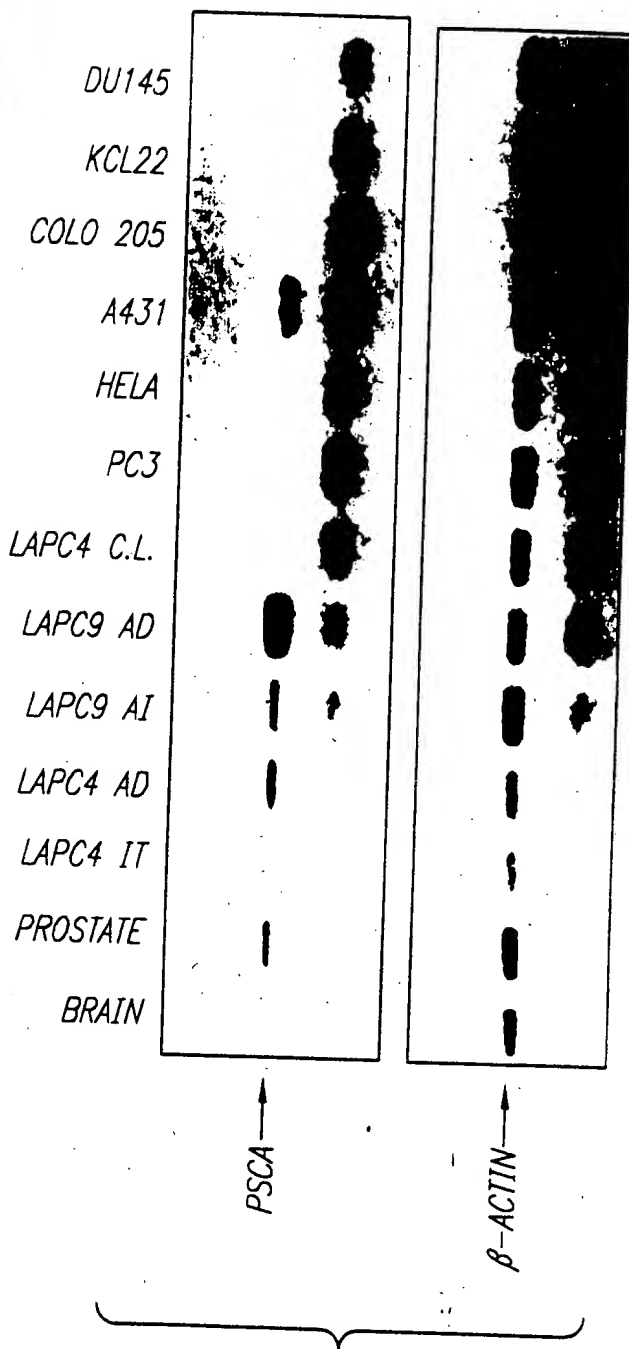
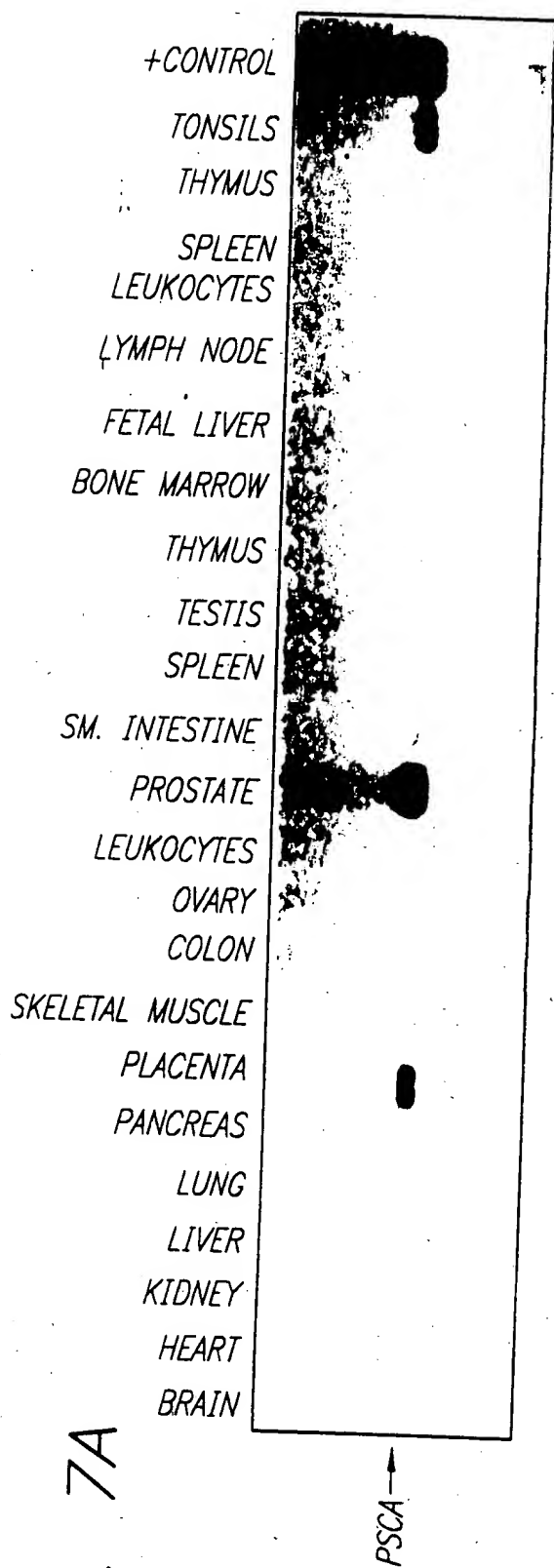
Western ASCA  
 Supposed to be 80% AB  
 Normal tissue  
 1hr exp

1G8  
 1:100



prostate (Humer)  
 prostate (Baker)  
 prostate (Gek)  
 Bladder (Humer)  
 Bladder (Gek)  
 Bladder (Rob)  
 Kidney (N1004)  
 Kidney (W121)  
 Testis  
 Sm. Intest.

LA PC9

FIGURE 6





Legend:  untranslated region of pSCA  
 translated region of pSCA

24-6/11/81

FIG. 8A

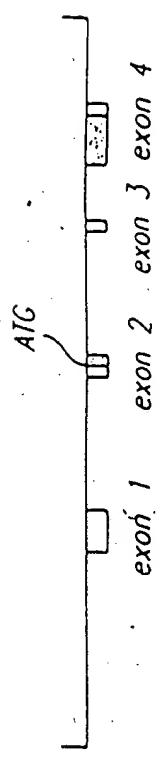


FIG. 8B

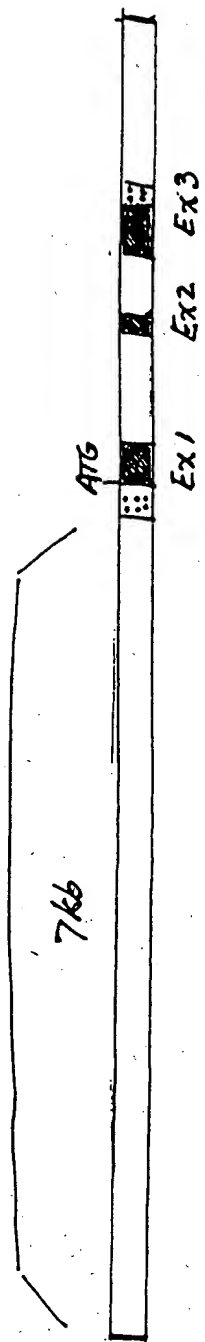
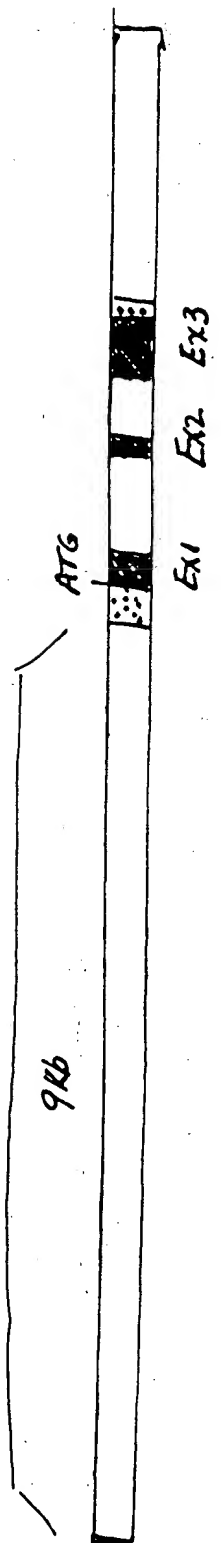


FIG. 8C



murine pSCA

FIGURE 8

human pSCA

PSCA / PSA Expression in Benign  
Prostate vs. Prostate Cancer Xenograft

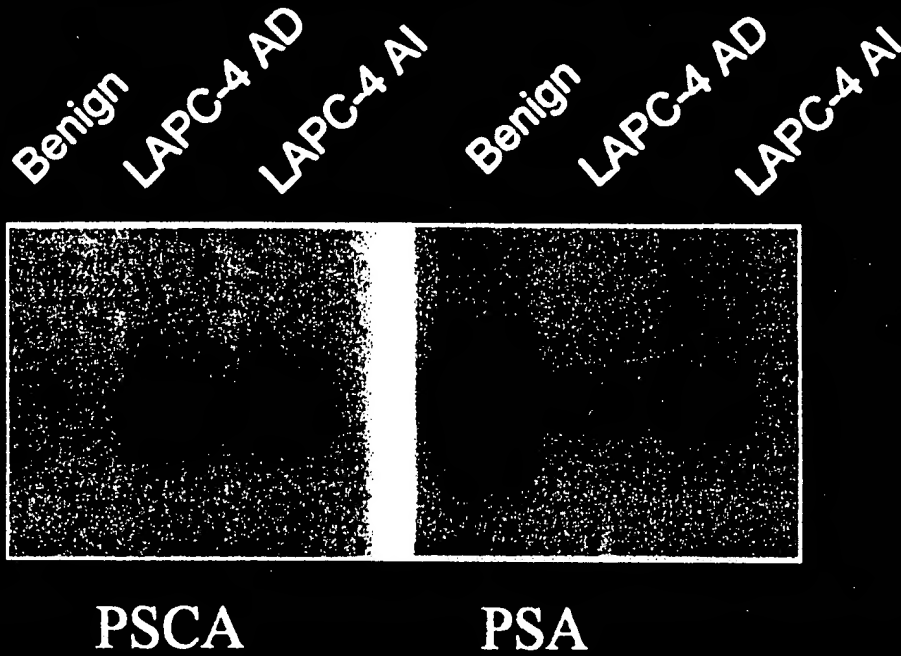


FIGURE 9A

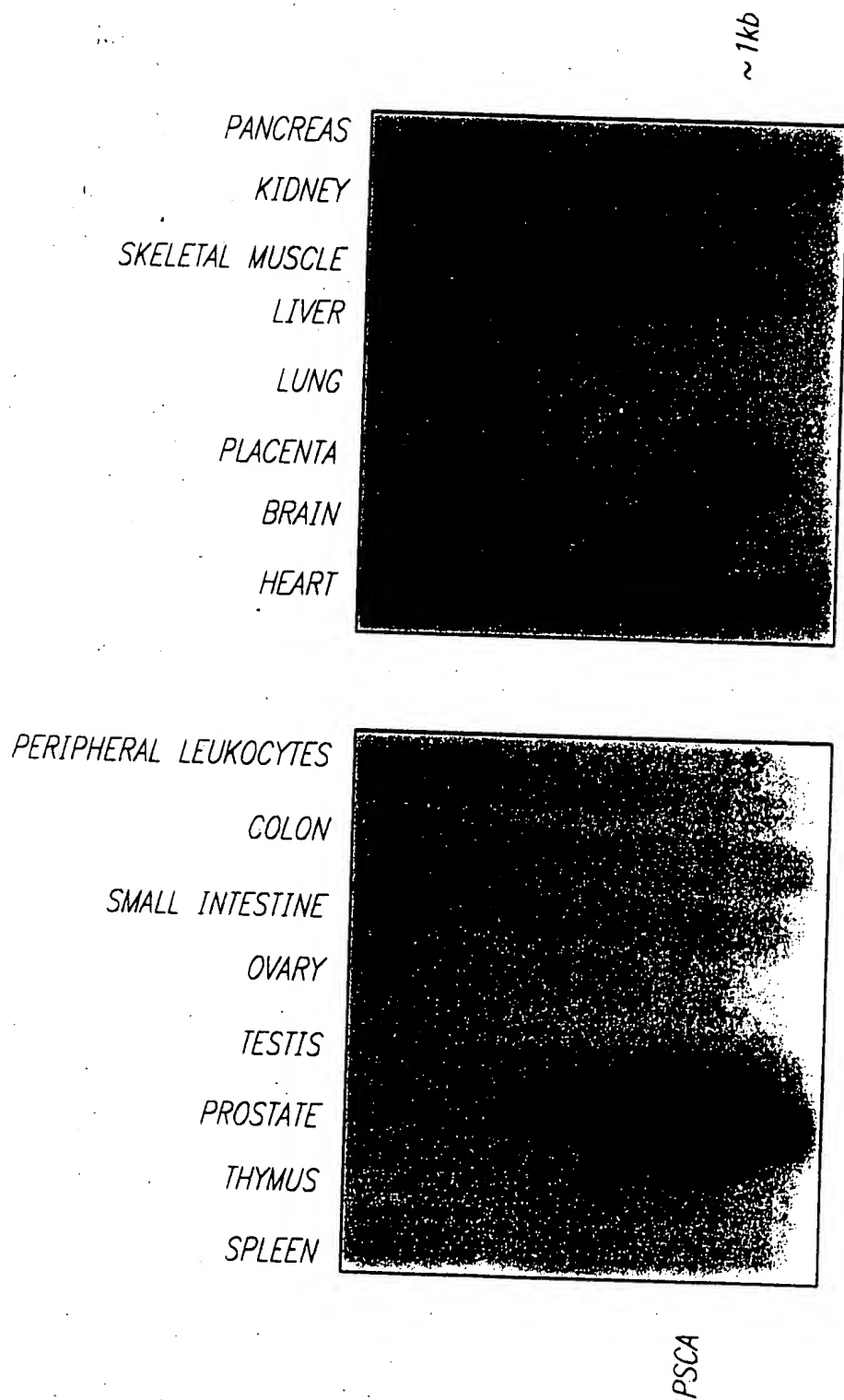
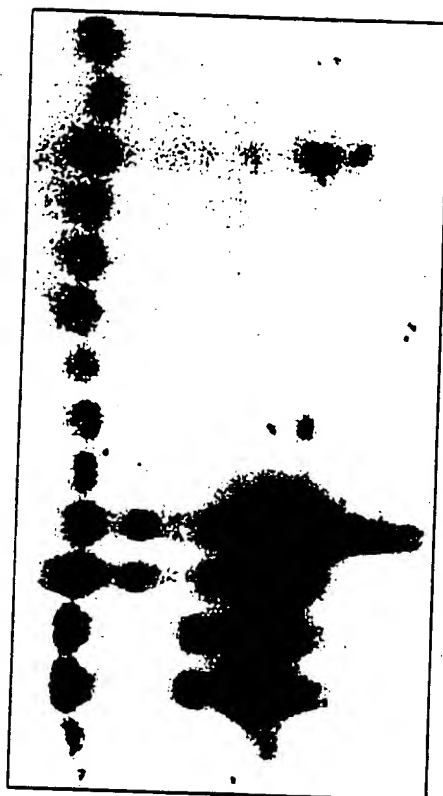


FIG. 9B

72 HRS

KCL22  
COLO 205  
A431  
HELA  
DU145  
PC3  
LNCAP  
LAPC4 C.L.  
LAPC3 AI  
LAPC9  
LAPC4 IT  
LAPC4 AI  
LAPC4 AD  
BPH



4 HRS

KCL22  
COLO 205  
A431  
HELA  
DU145  
PC3  
LNCAP  
LAPC4 C.L.  
LAPC3 AI  
LAPC9  
LAPC4 IT  
LAPC4 AI  
LAPC4 AD  
BPH

PSCA

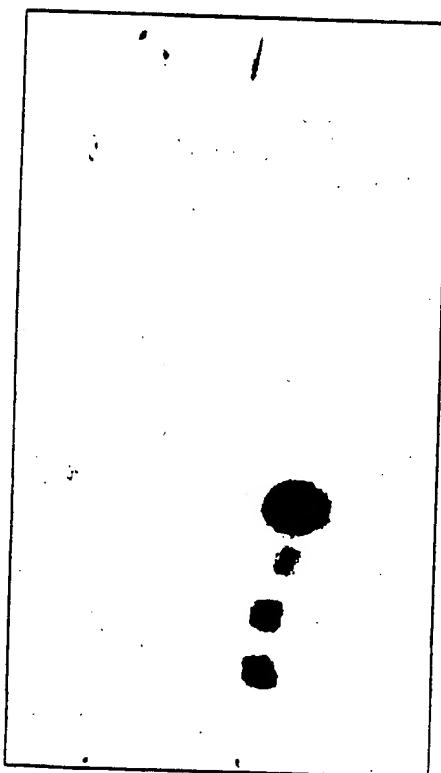
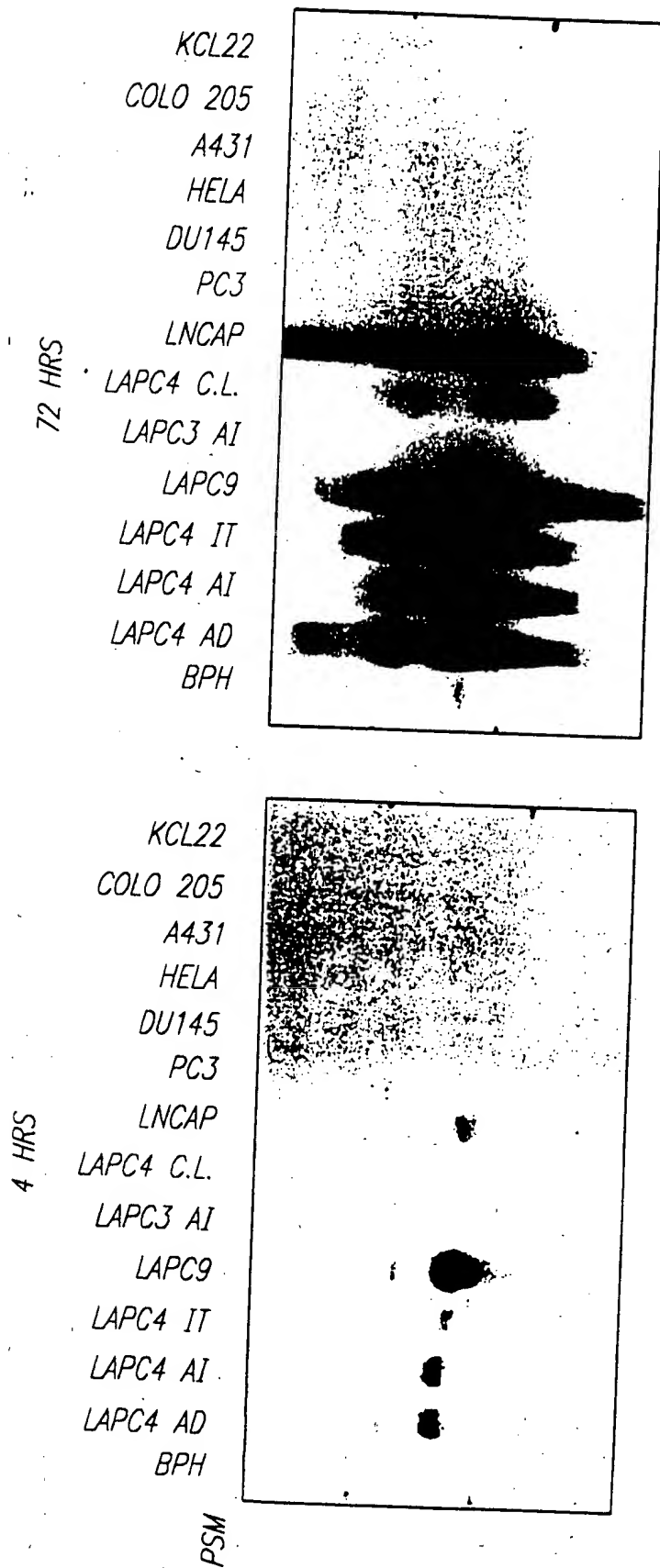


FIG. 10-1



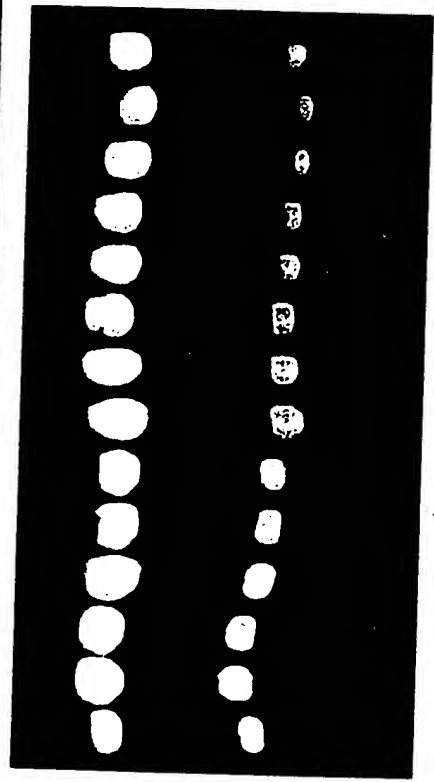
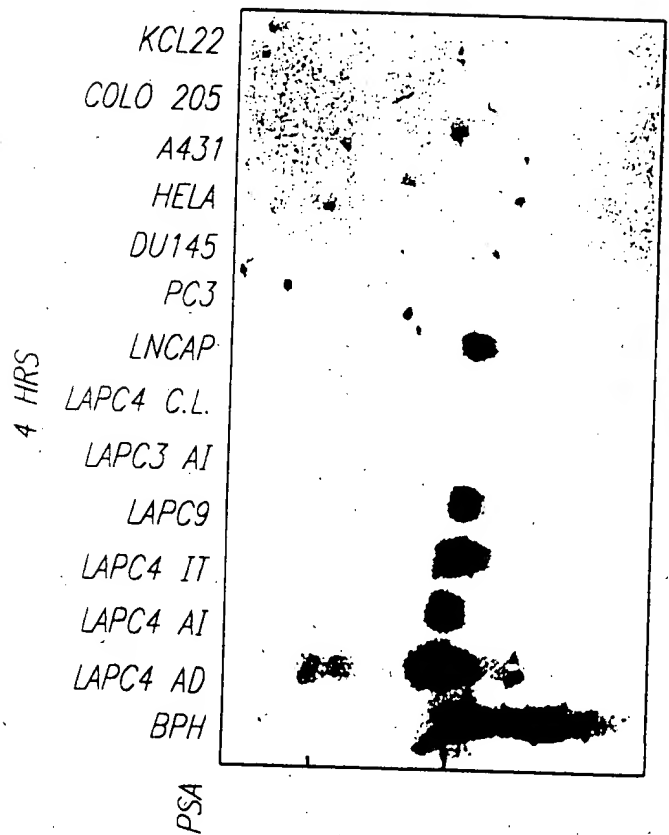
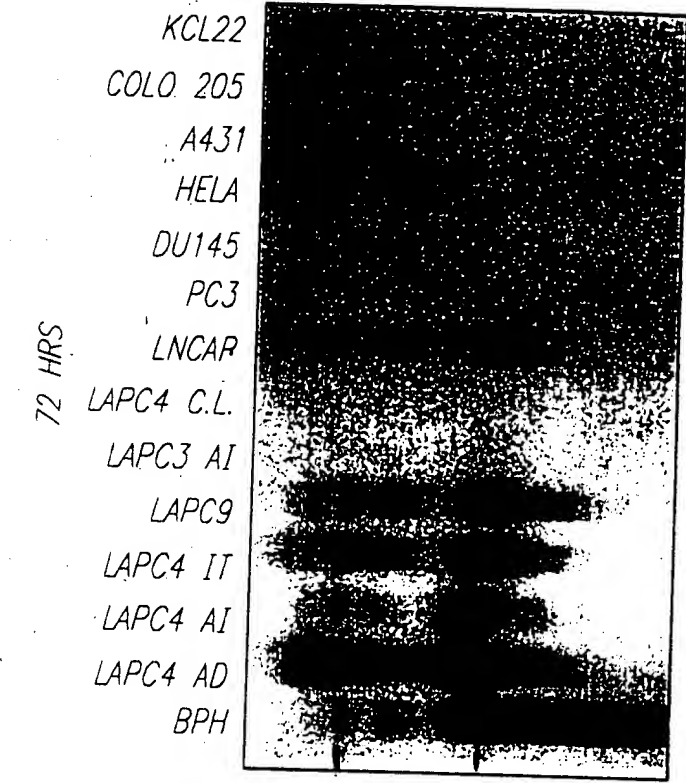


FIG. 10-3

FIG. 11A



FIG. 11B

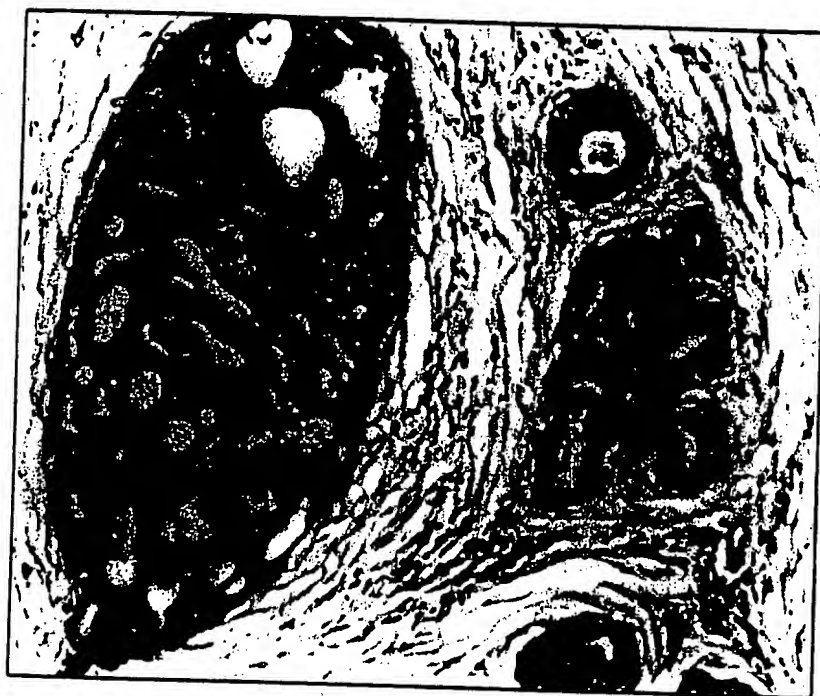


FIG. 11C



FIG. 12A

CONTROL  
N GLYCOSIDASE F  
O GLYCOSIDASE

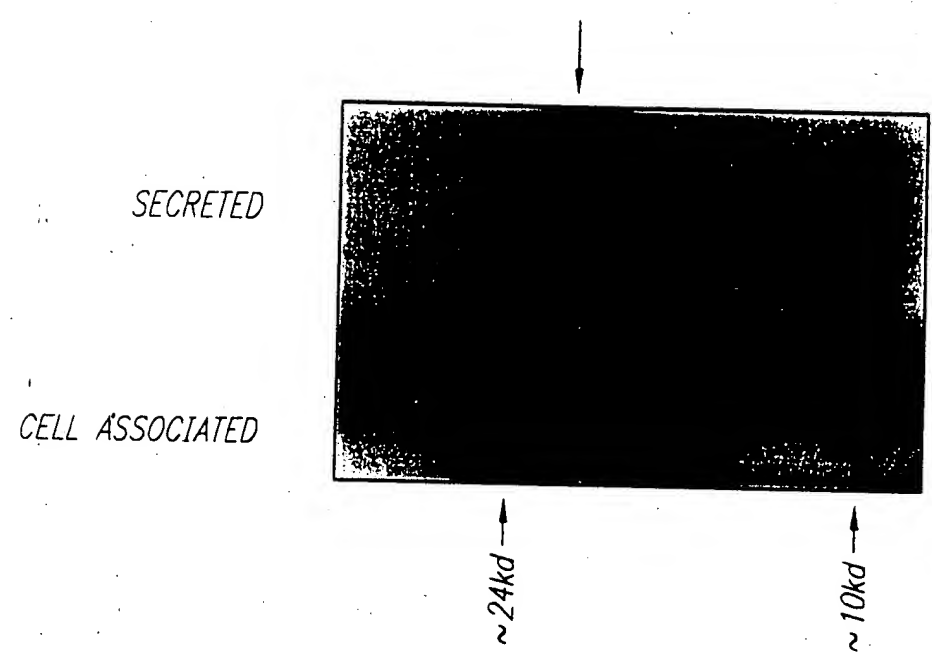
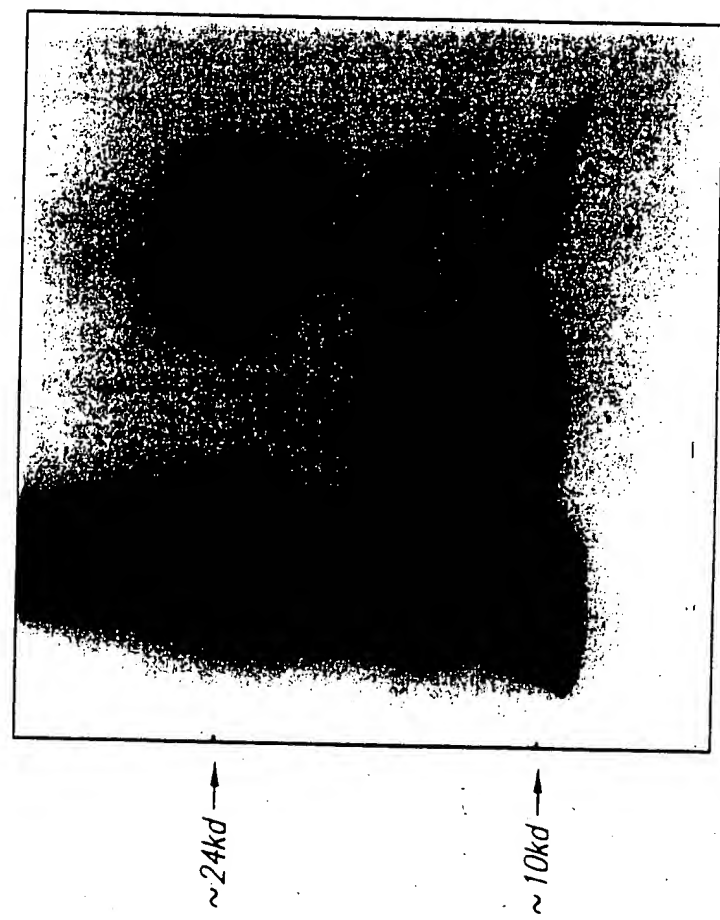


FIG. 12B

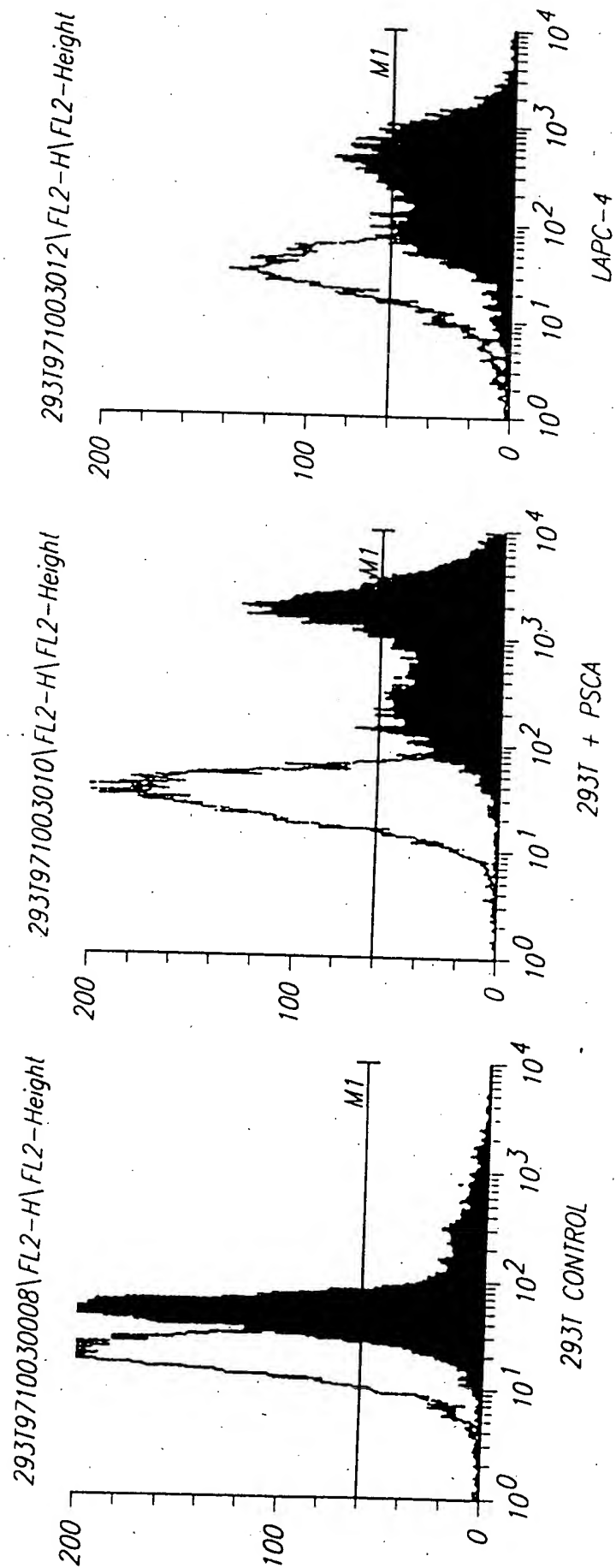


FIGURE 12C

## PSCA Maps to Chromosome 8q24.2



Fluorescent  
in Situ Hybridization  
Analysis of PSCA

FIGURE 13

0955105100 EST 5860

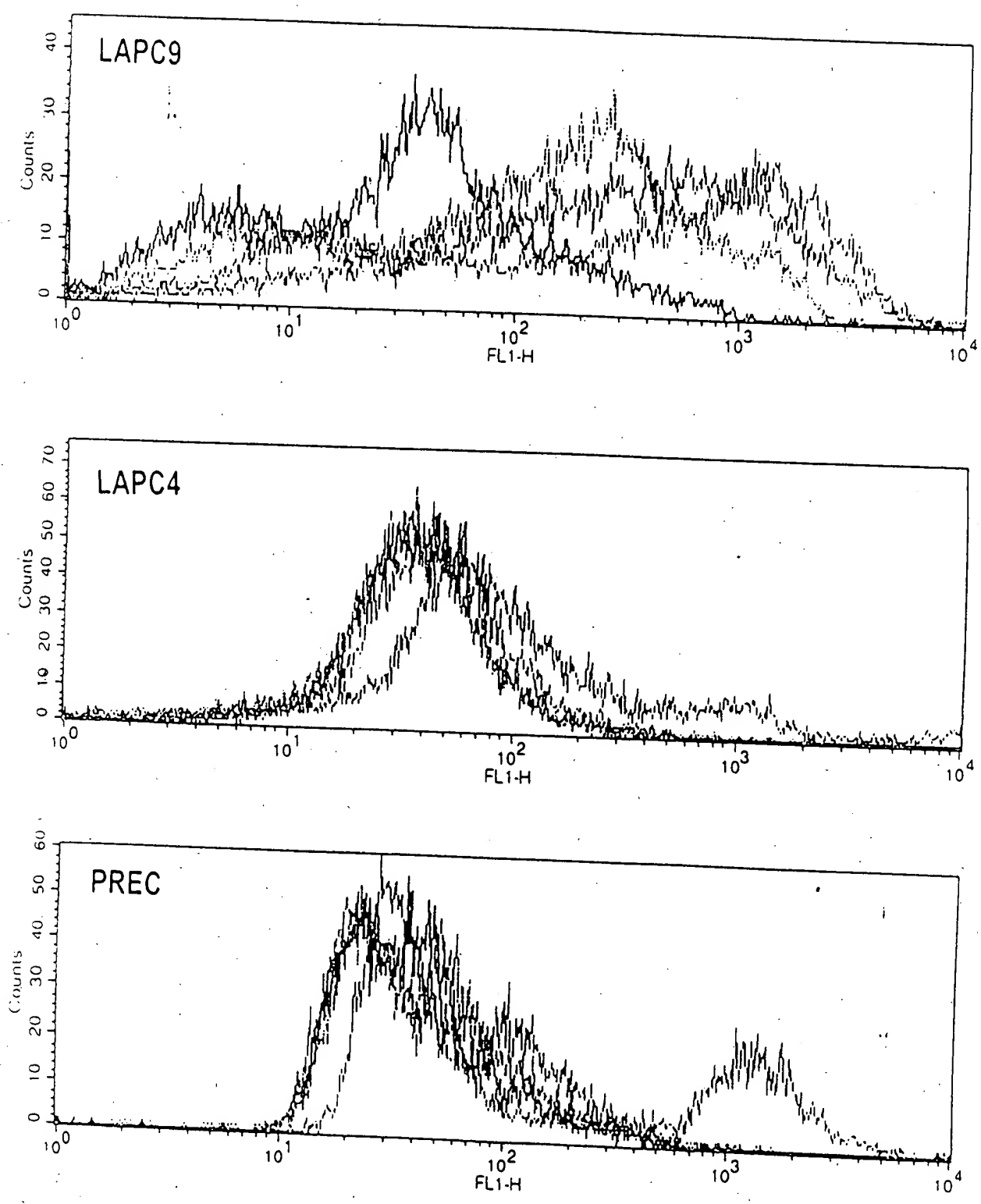


FIGURE 14

**A**

**Epitope map**

mAb	Isotype	EL (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	2.039	0.007	0.628	0.000
2H9	IgG1 k	1.318	0.863	0.032	0.021
3C5	IgG2a k	2.893	1.965	0.016	0.005
3E6	IgG3 k	0.328	0.024	0.069	0.370
4A10	IgG2a k	2.039	1.315	0.000	0.014
2A2	IgG2a k	1.366	0.733	0.010	0.003
3G3	IgG2a k	2.805	1.731	0.004	0.000

**B**

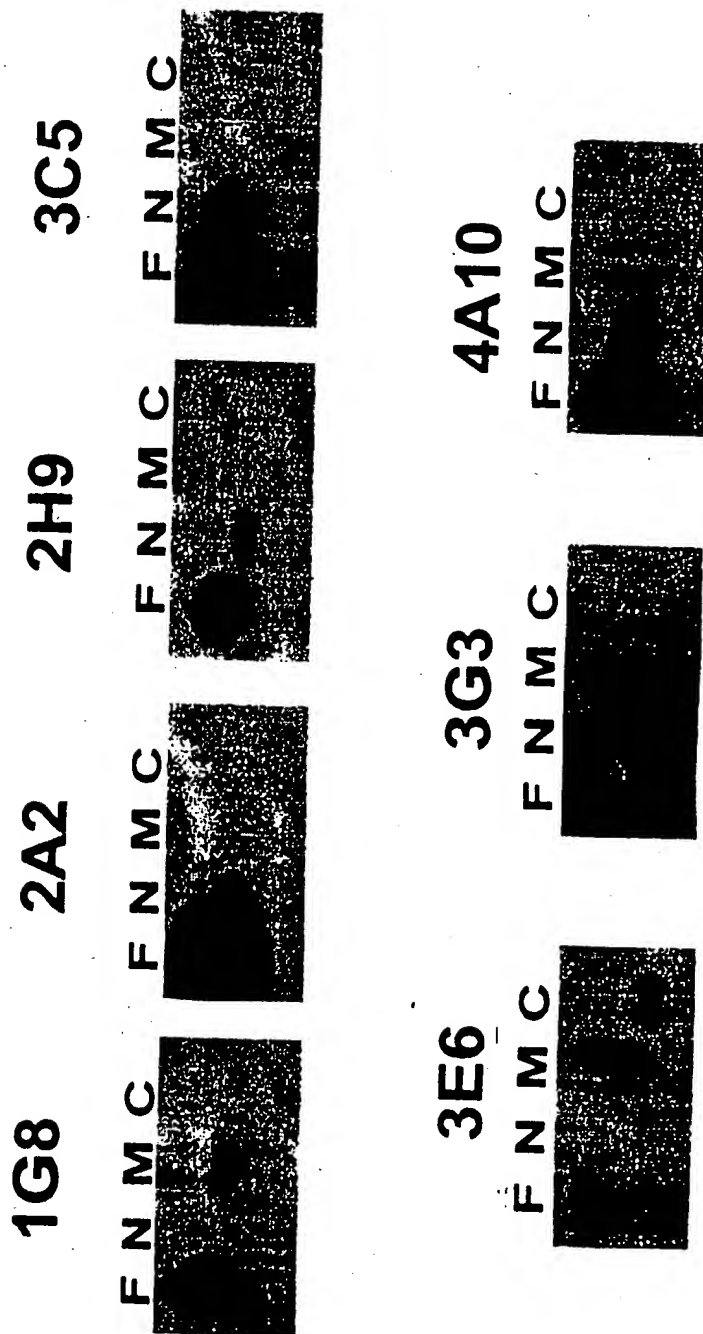


FIGURE 15

096571

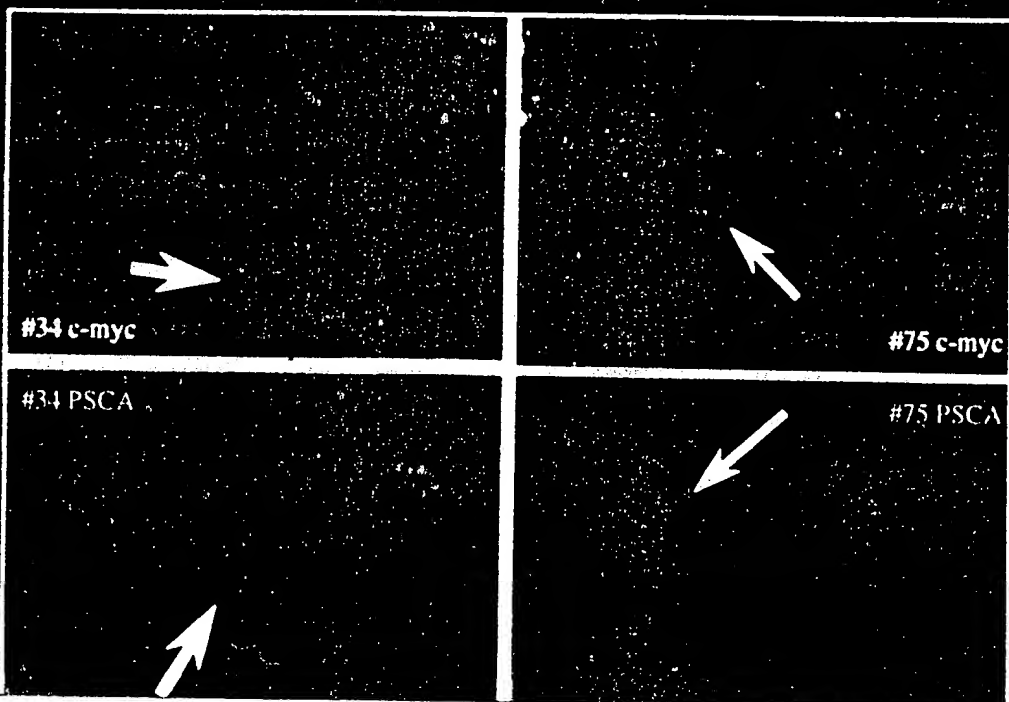
(Reiter, R.E., et al., 1997, *PNAS*)



# FISH Analysis of PSCA and c-myc in Prostate Cancer

Gain Chromosome 8

Amplification



*R. Jenkins*

FIGURE 17

09855153-051401



FIGURE 18



0985153 051401  
FOI 50 " CTS 50

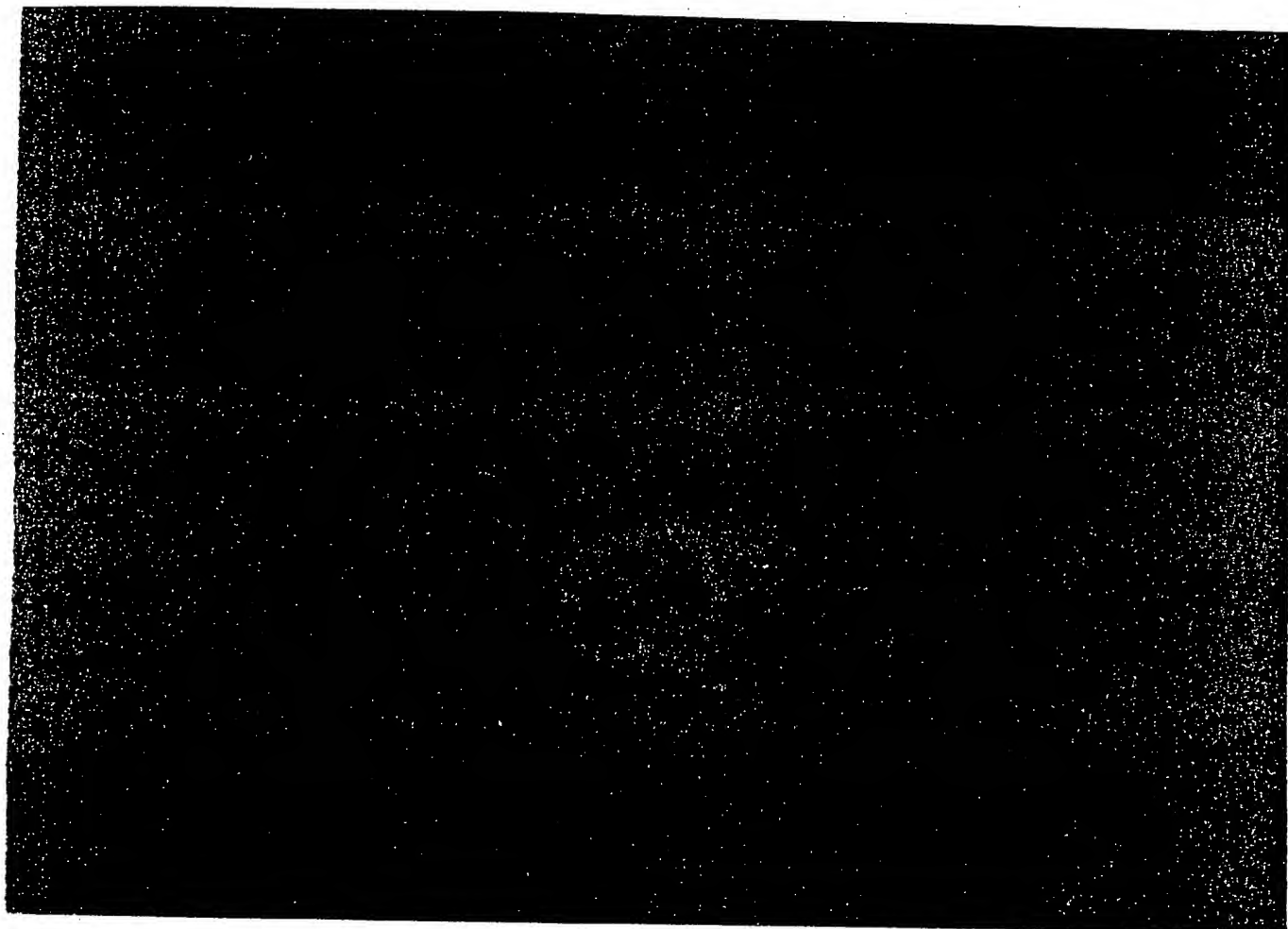


FIGURE 19

09855153-051401



FIGURE 20

## PSCA Immunostaining of Primary Tumors

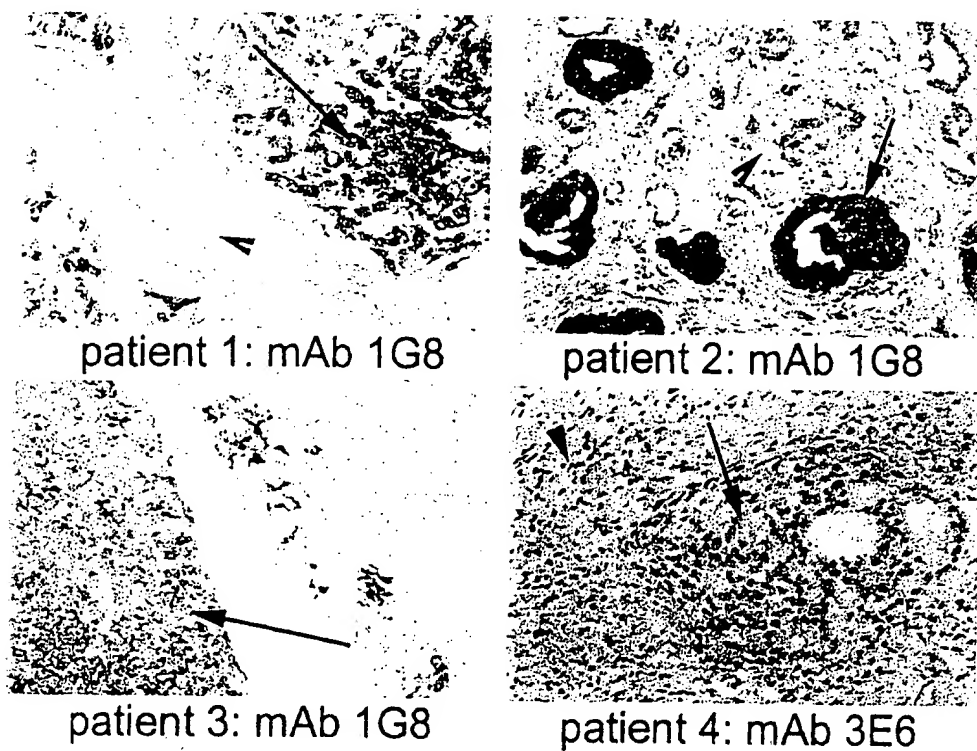


FIGURE 21

0955153-051401



FIGURE 22

0985153-051401



FIGURE 23

09855153-051401



FIGURE 24

1. 1000  
 2. 1000  
 3. 1000  
 4. 1000  
 5. 1000  
 6. 1000  
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 96. 1000  
 97. 1000  
 98. 1000  
 99. 1000  
 100. 1000

FIGURE 25

09855153-051401

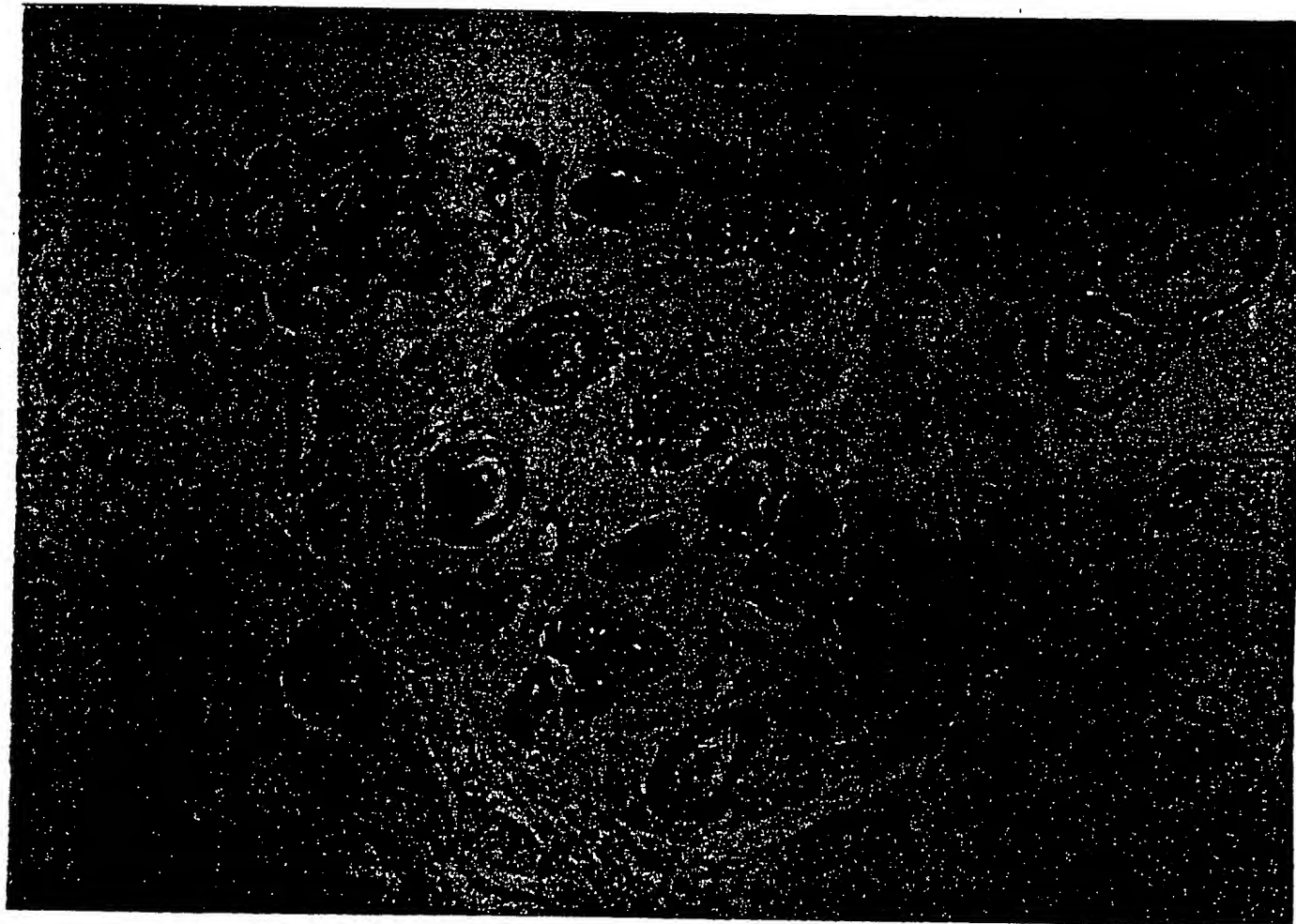


FIGURE 26



FOUO EST 5860

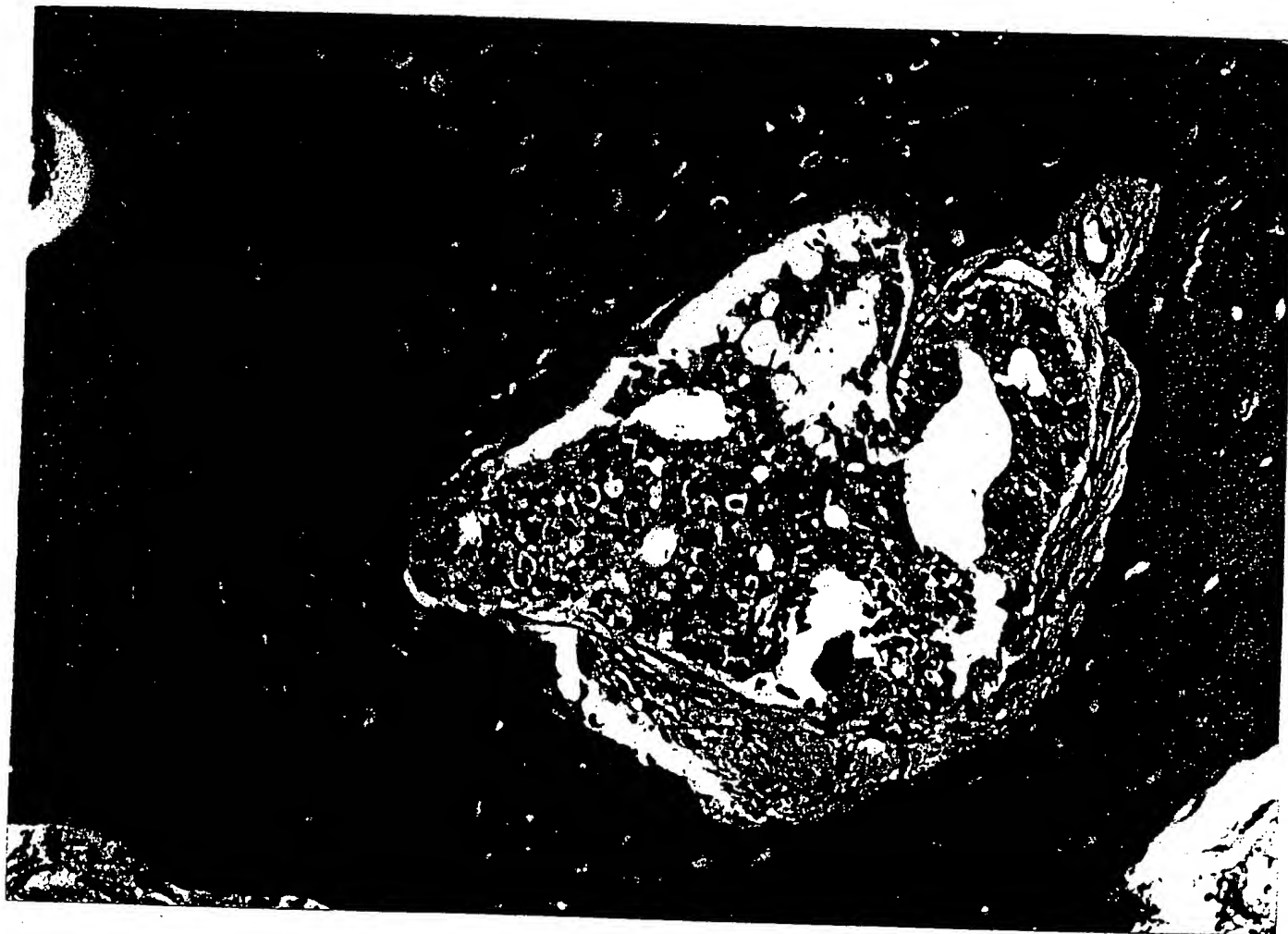
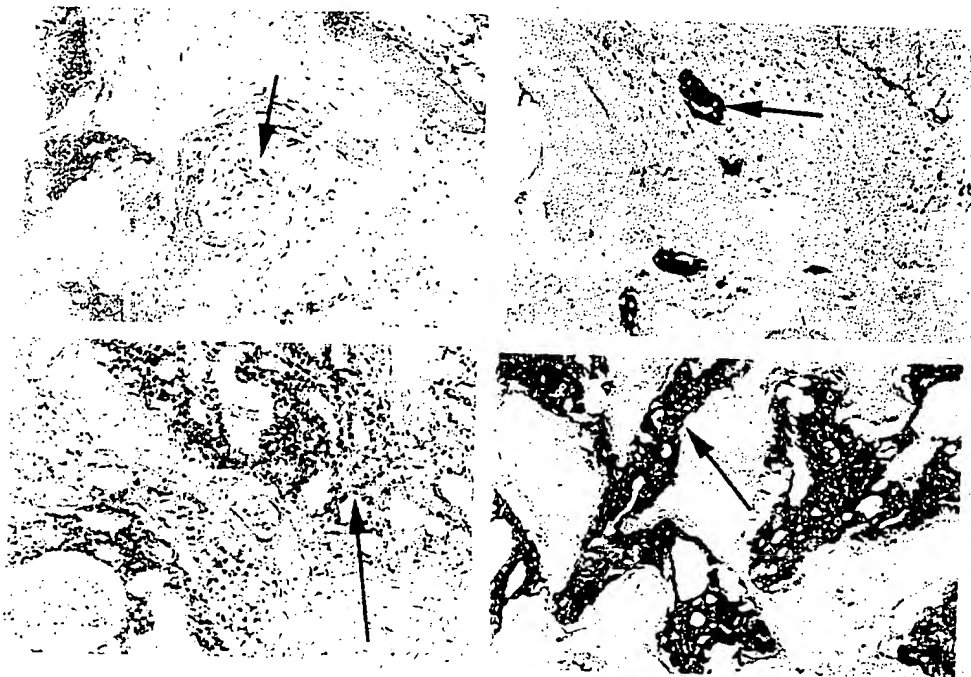


FIGURE 27

## PSCA Immunostaining of Bony Metastases



Patient 5: H and E  
and mAb 1G8

Patient 4: H and E  
and mAb 3E6

FIGURE 28

FOOTSTOCK ESTABLISHMENT

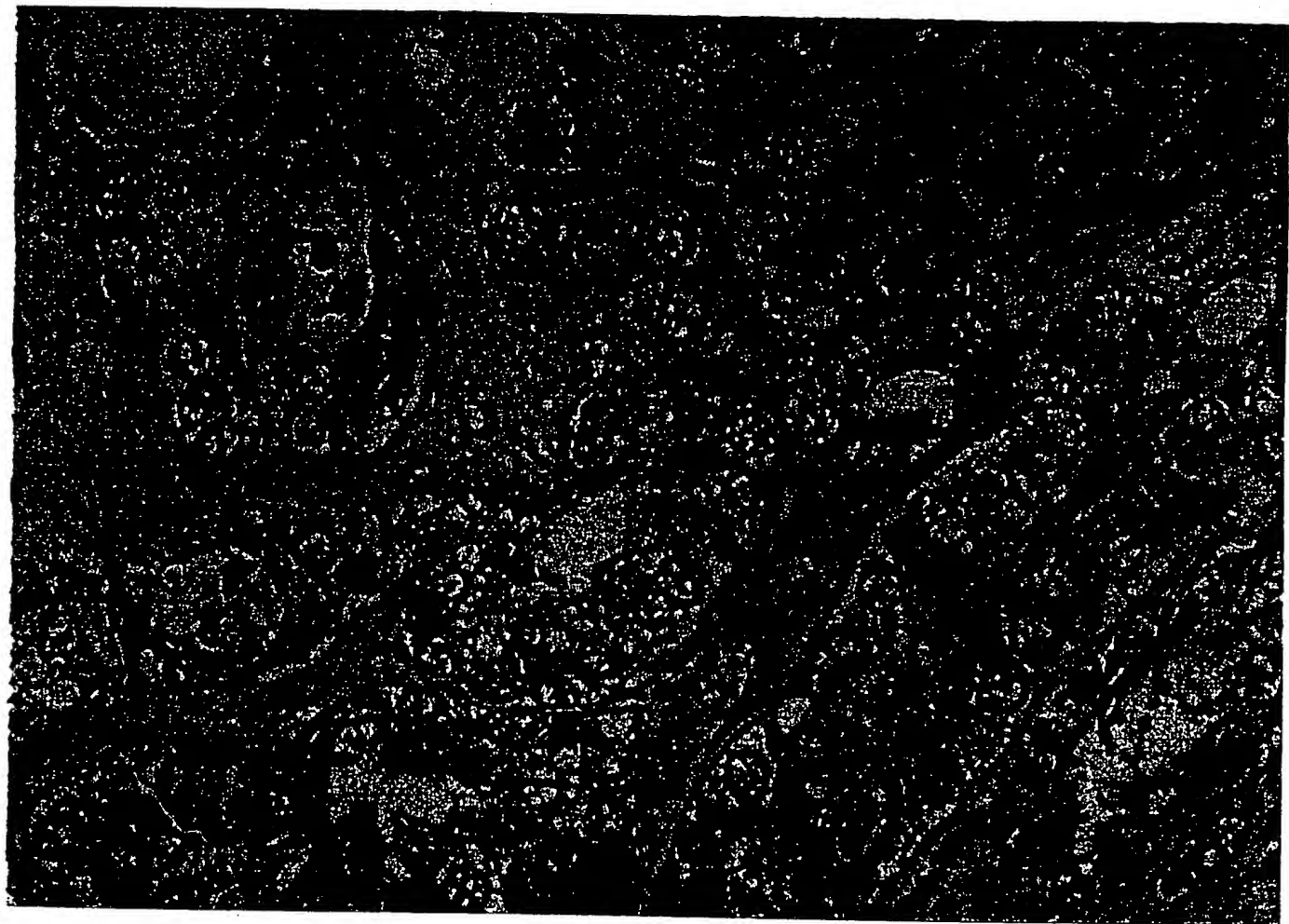


FIGURE 29

09855153-051401

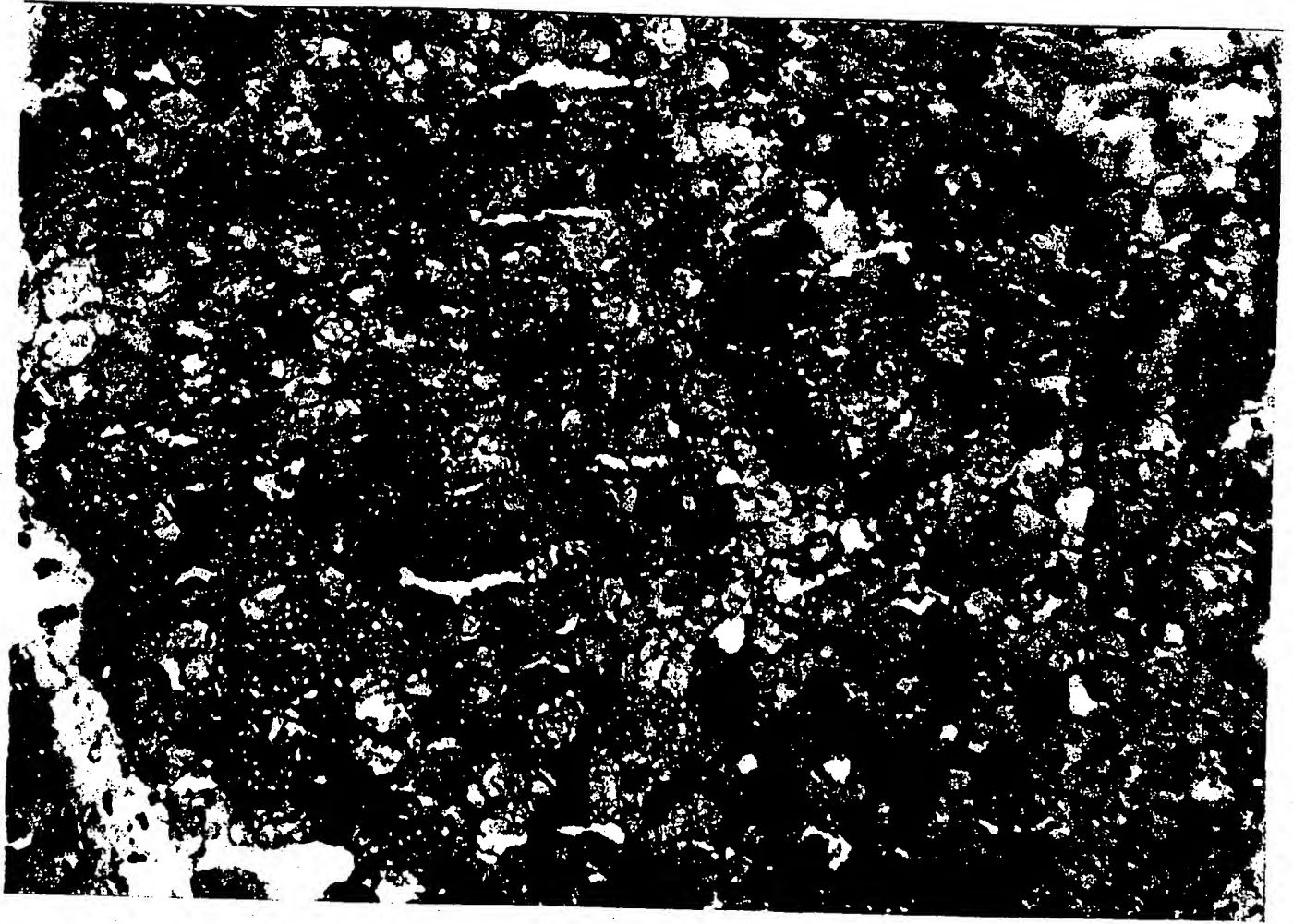


FIGURE 30

09855153-051401

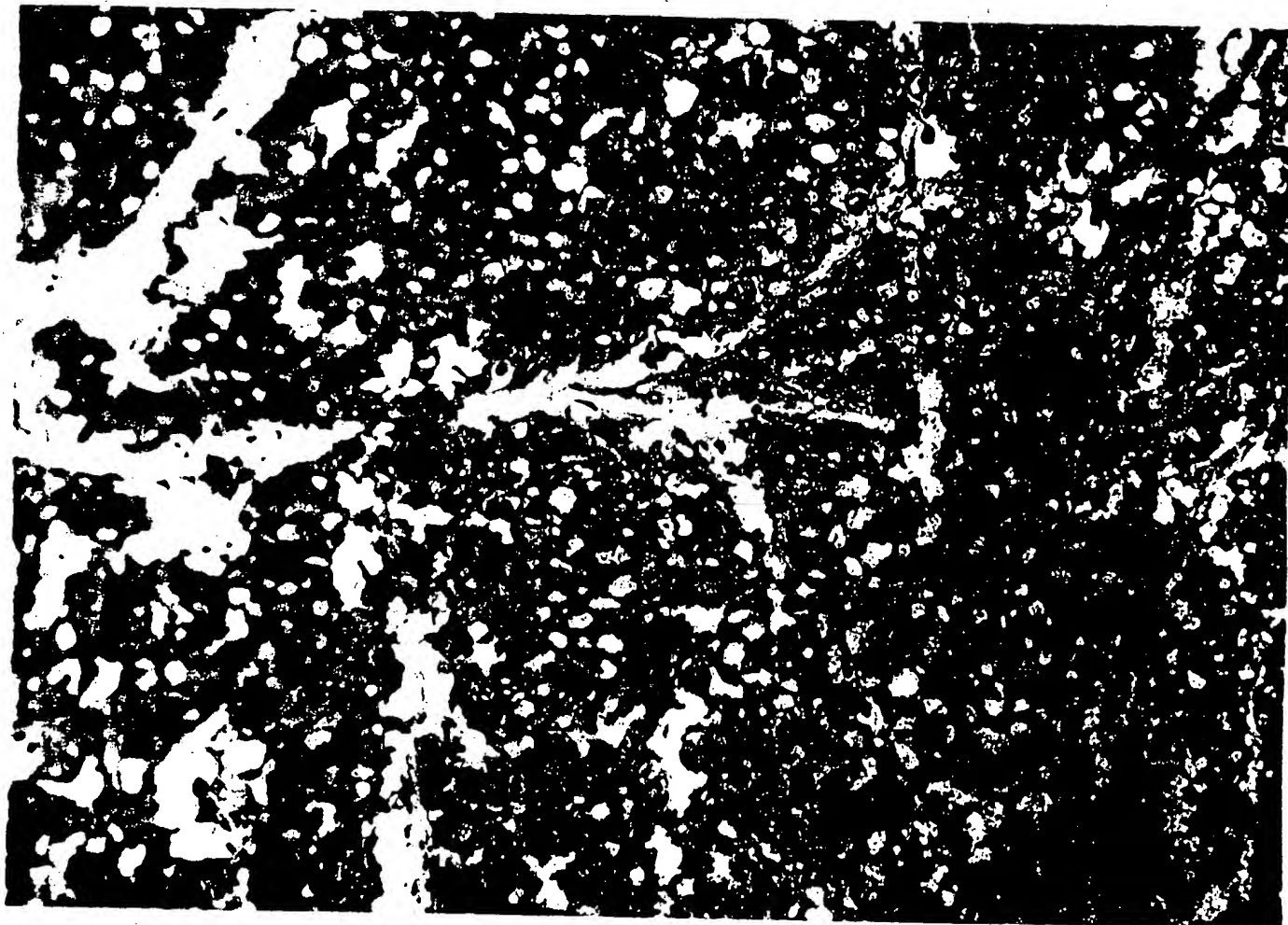


FIGURE 31

0985153-051401

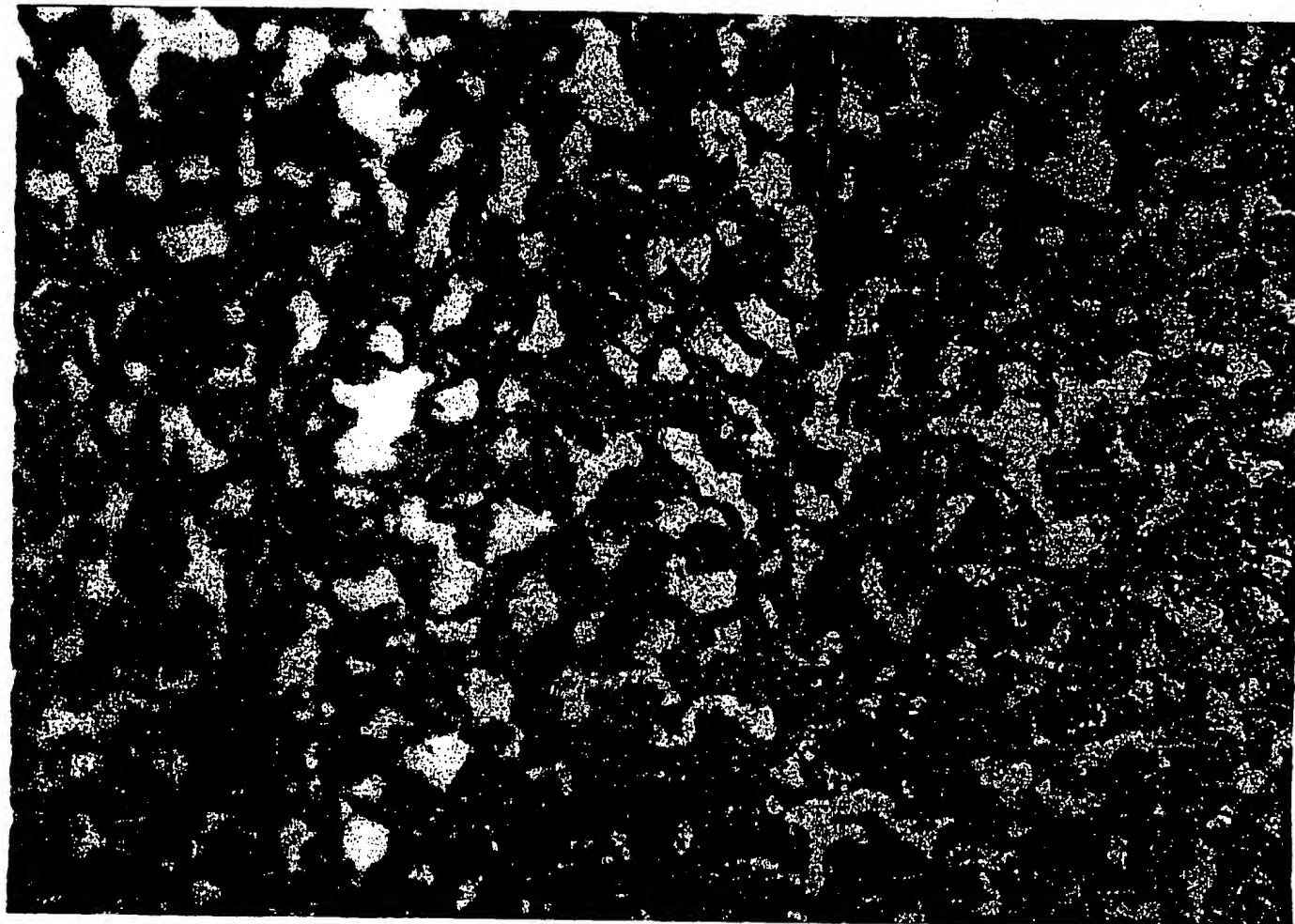


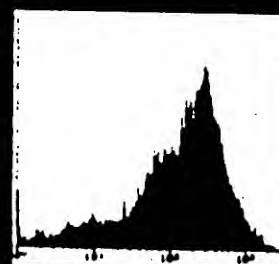
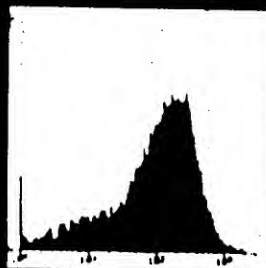
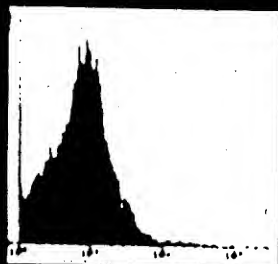
FIGURE 32

# PSCA Expression in LAPC-9 Xenograft by FACS

Secondary Antibody

1G8

2H9



4A10

3C5

3E6

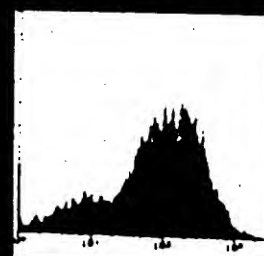
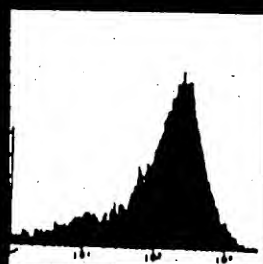
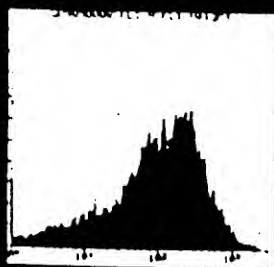


FIGURE 33

09551540 EST5860

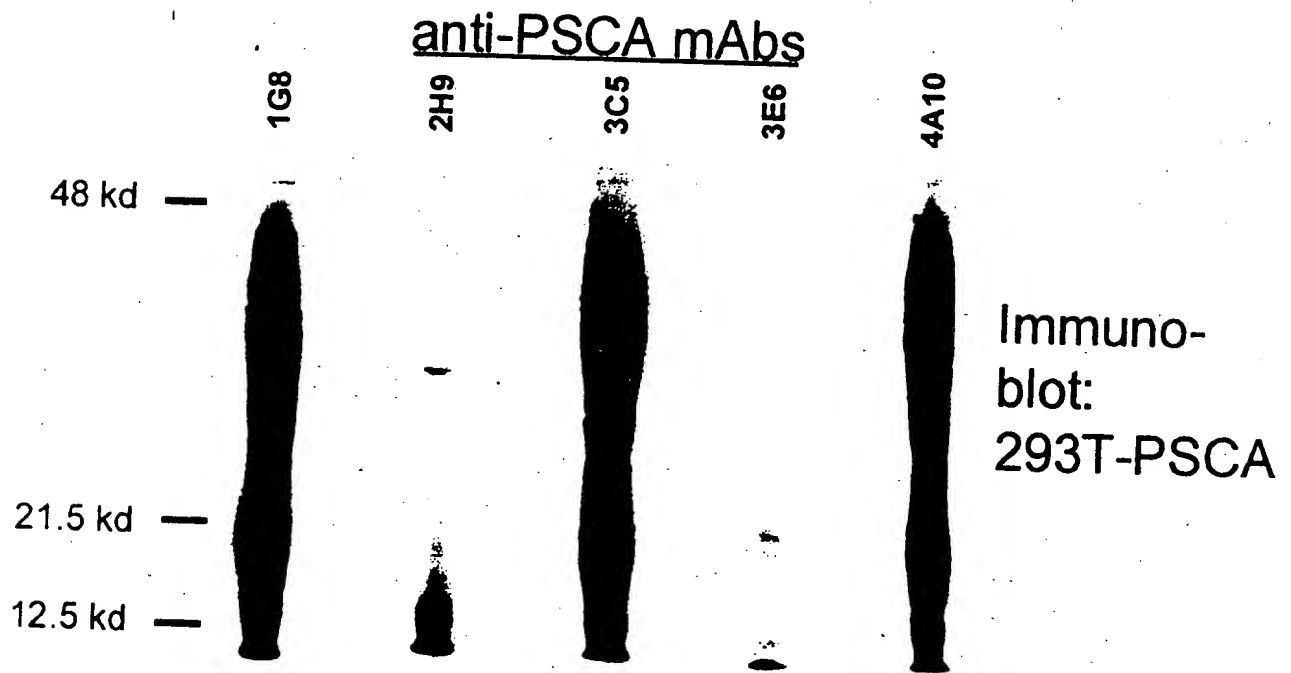


FIGURE 34



## Immunofluorescent Staining of LNCaP-PSCA Cells

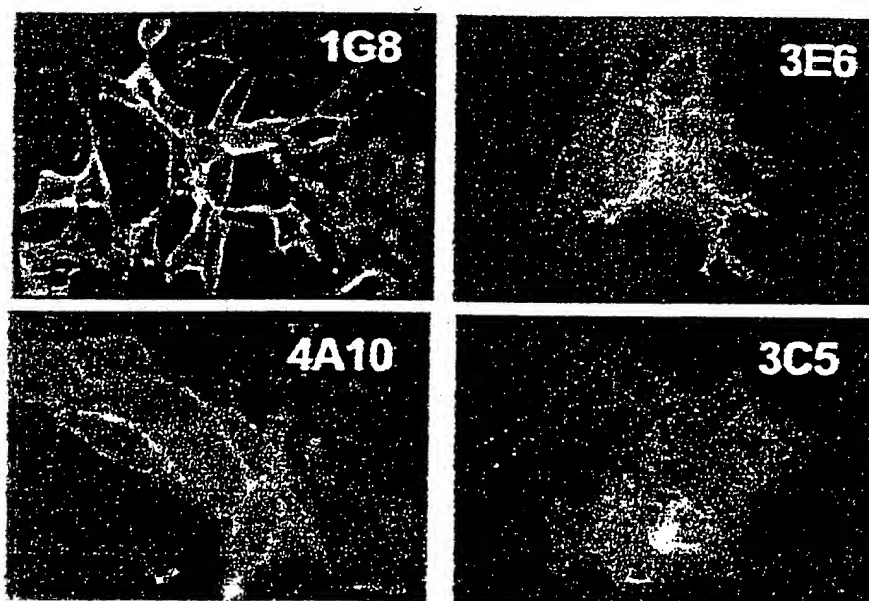


FIGURE 35

0955153.051401  
FOH T50" EST5860

TOP SECRET

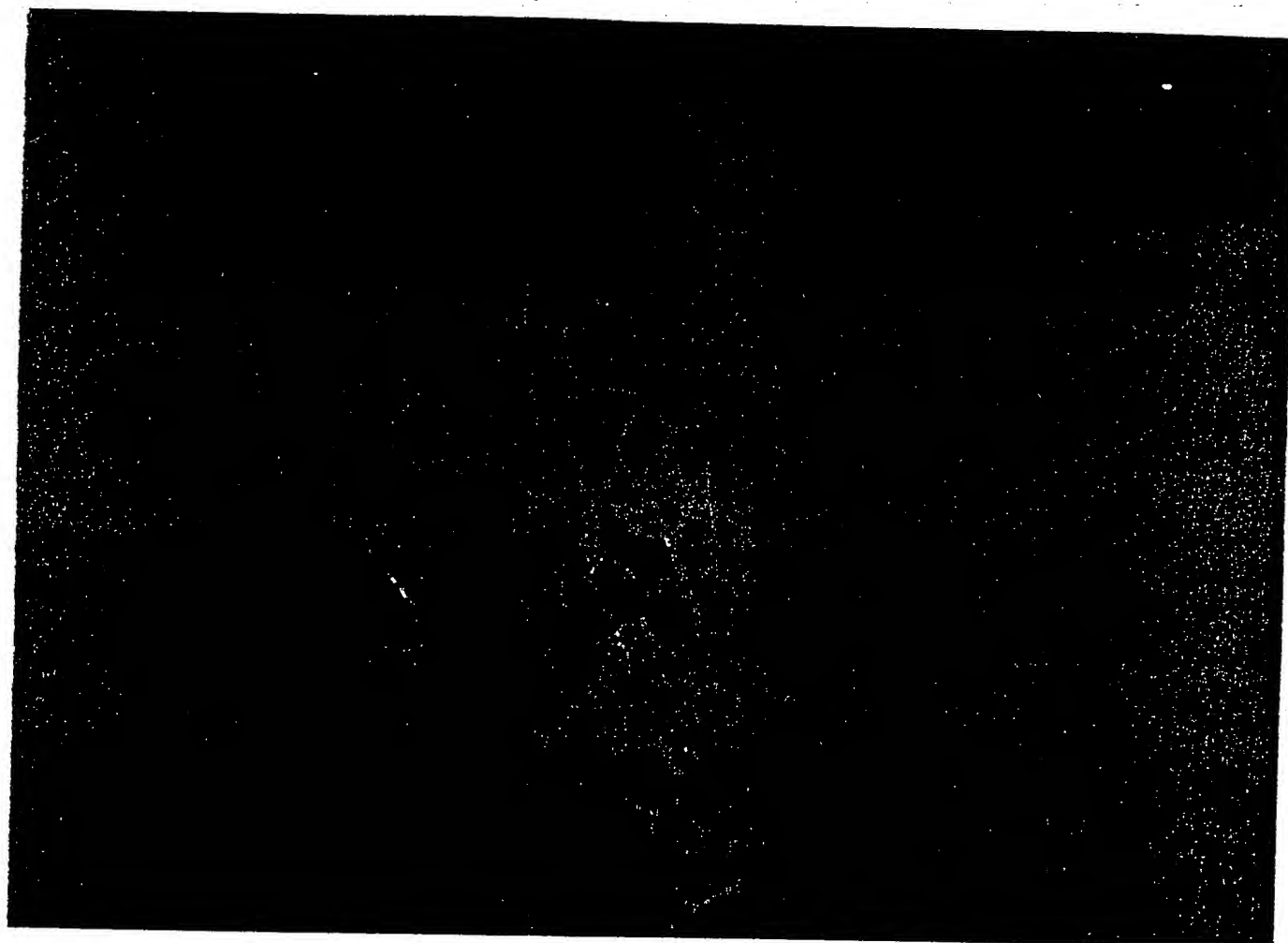


FIGURE 36

204T50" EST5860

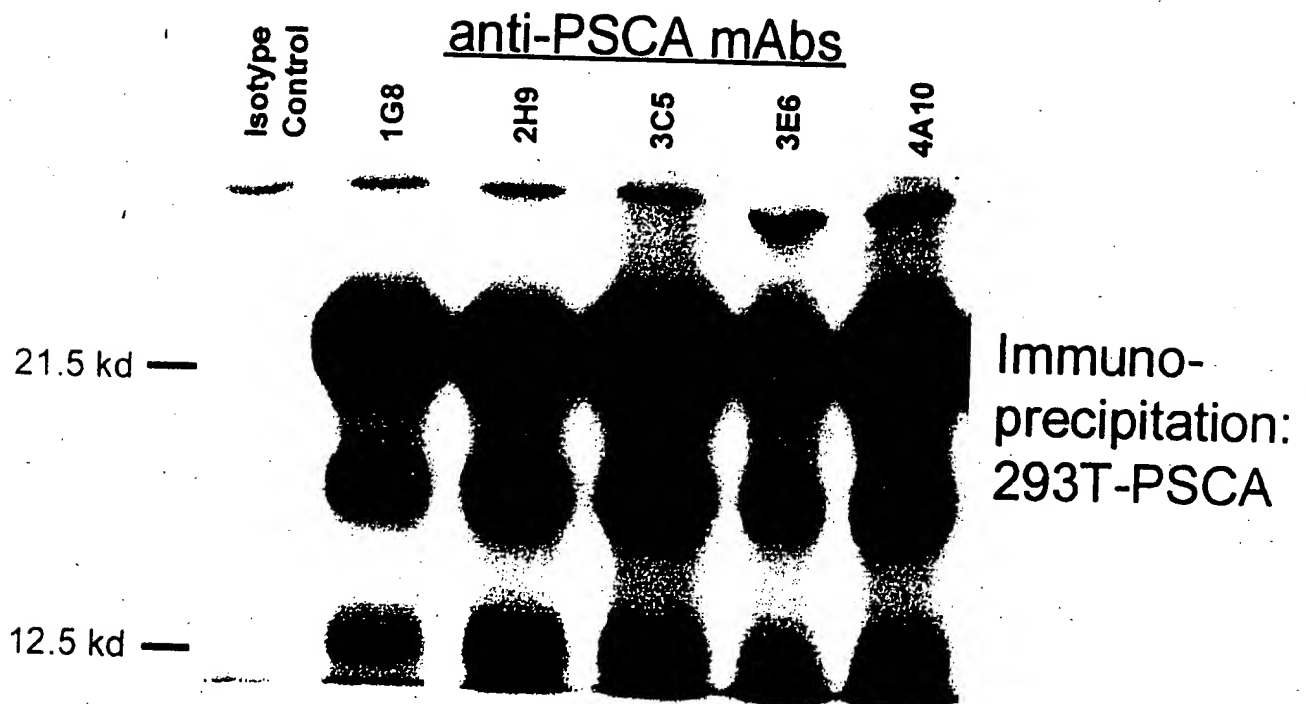


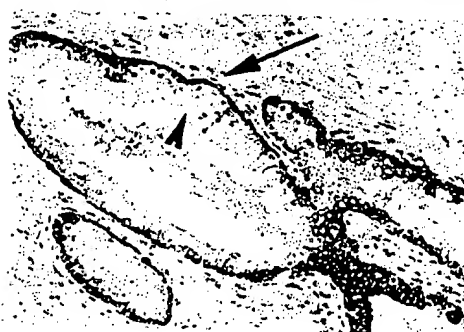
FIGURE 37

## Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control



Normal: PSCA mAb 3E6



Normal: PSCA mAb 1G8



Atrophy: PSCA mAb 2H9

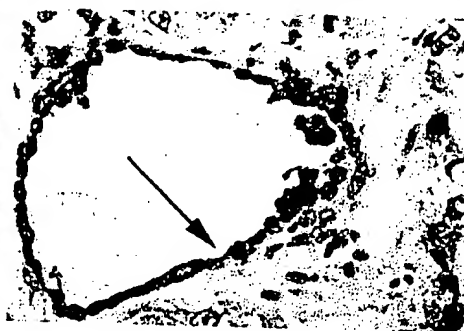
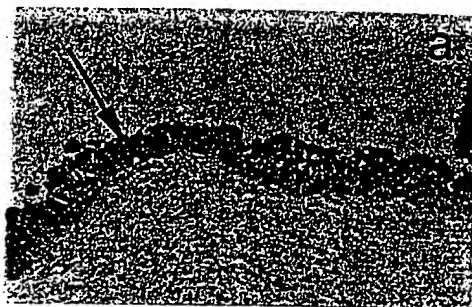
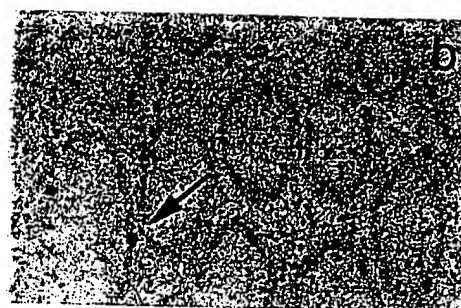


FIGURE 38

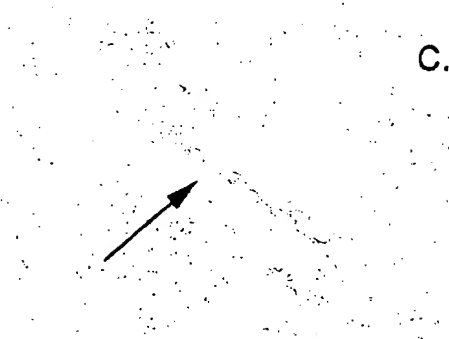
A.



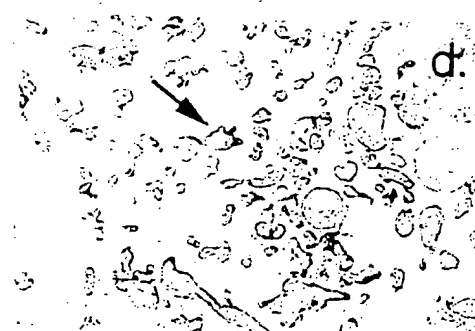
Bladder: 1G8



Colon: 1G8

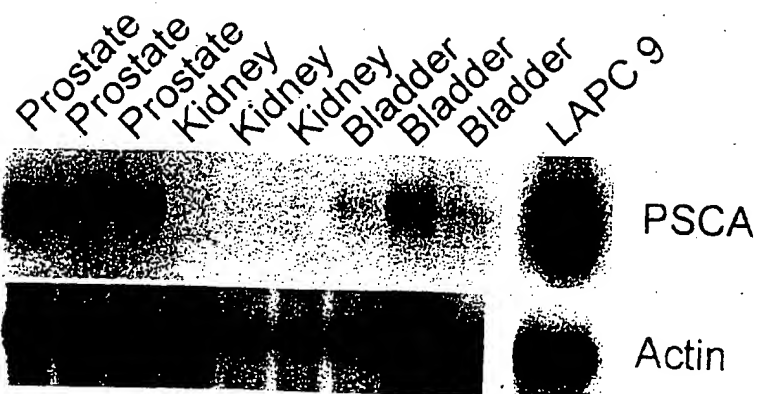


Kidney: 3E6



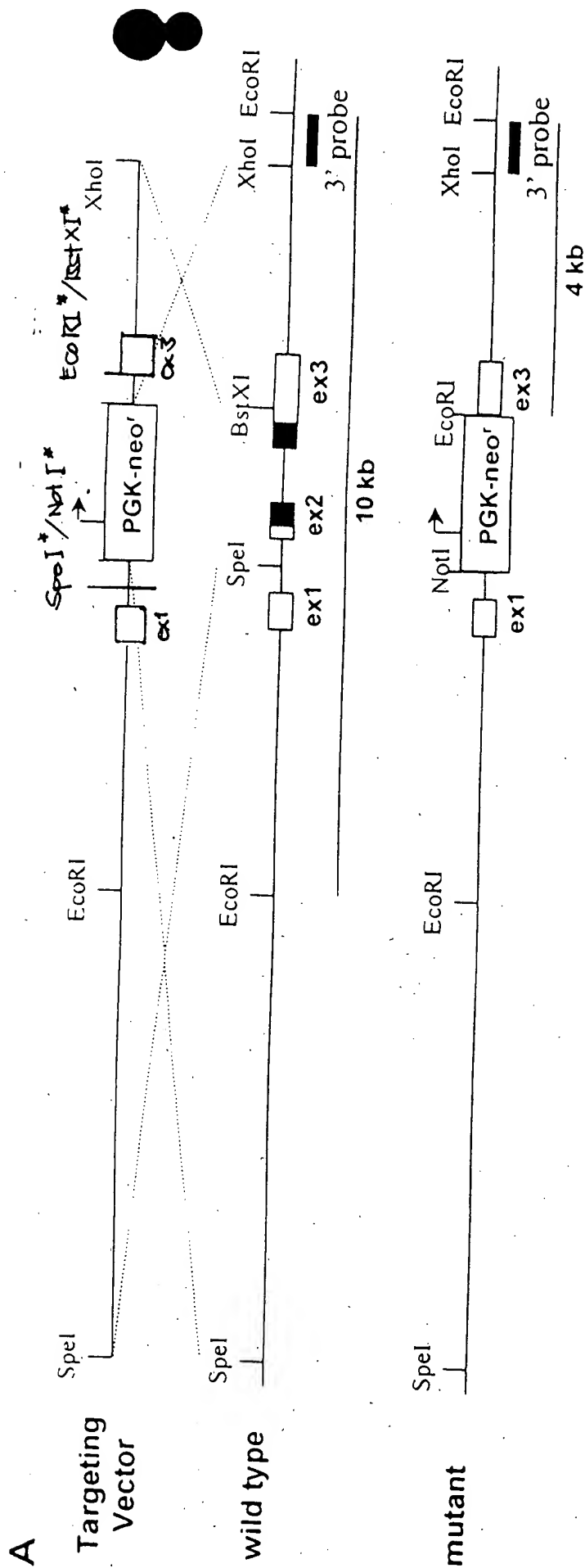
Placenta: 3E6

B.



**FIGURE 39**

# Targeting of Mouse PSCA Gene



- \* ex1, 2, and 3 are the exons of PSCA gene.
- \* Black boxes of ex2 and ex3 encode PSCA mature protein sequences.
- \* ES genomic DNA's were digested with EcoRI, followed by Southern hybridization using 3' probe.

## B. Genomic Southern Analysis of ES Cells

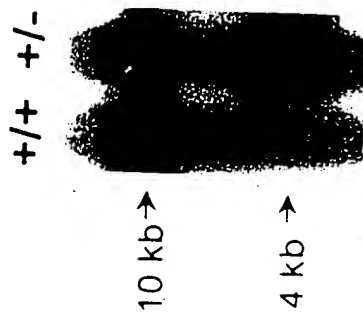
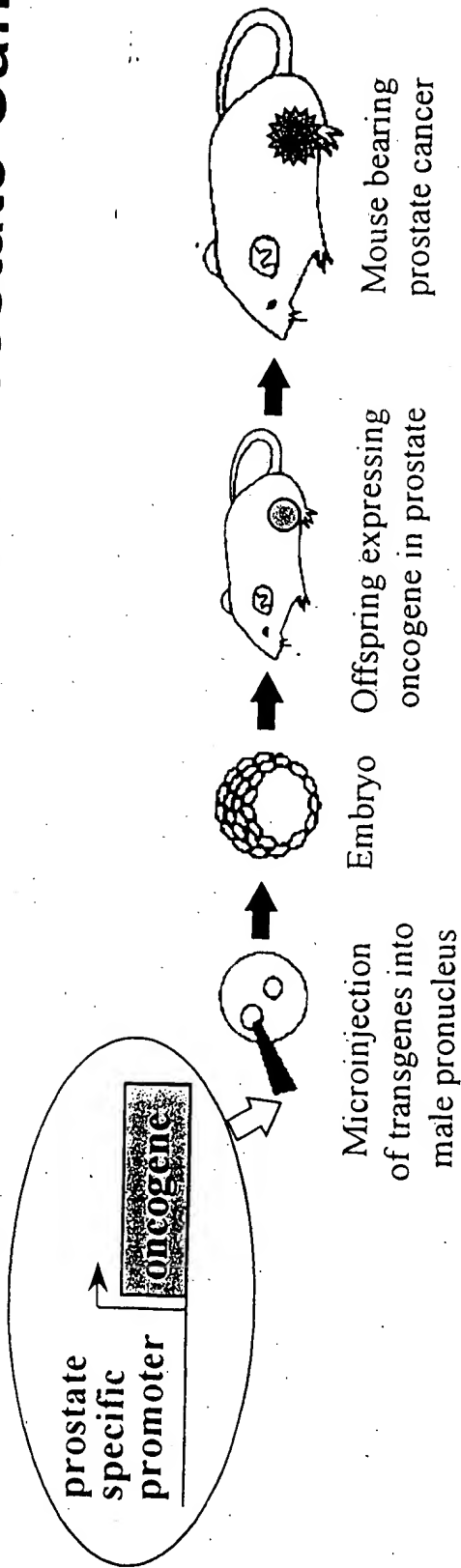


FIGURE 40

# Transgenic Mouse Models of Prostate Cancer



Transgene	Target tissues	Characteristics
C3(1) (-3 kb)/SV40 large+small, <i>Maroulakou et al. 1994 PNAS</i>	prostate (secretory cells) urethral, mammary and sweat gland	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 28 wks No metastases
Probasin (-426 bp)/SV40 large+small, <i>Greenberg et al. 1995 PNAS</i>	prostate (secretory cells)	Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcinoma 12 wks Metastases in lymph node, lung, liver and bone
Cryptdin2 (-6.5 kb)/SV40 large+small, <i>Garabedian et al. 1998 PNAS</i>	prostate (neuroendocrine cells) small intestine	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcinoma 16 wks Metastases in lymph node, lung, liver and bone

# Reporter Gene Constructs for Transfection Assay

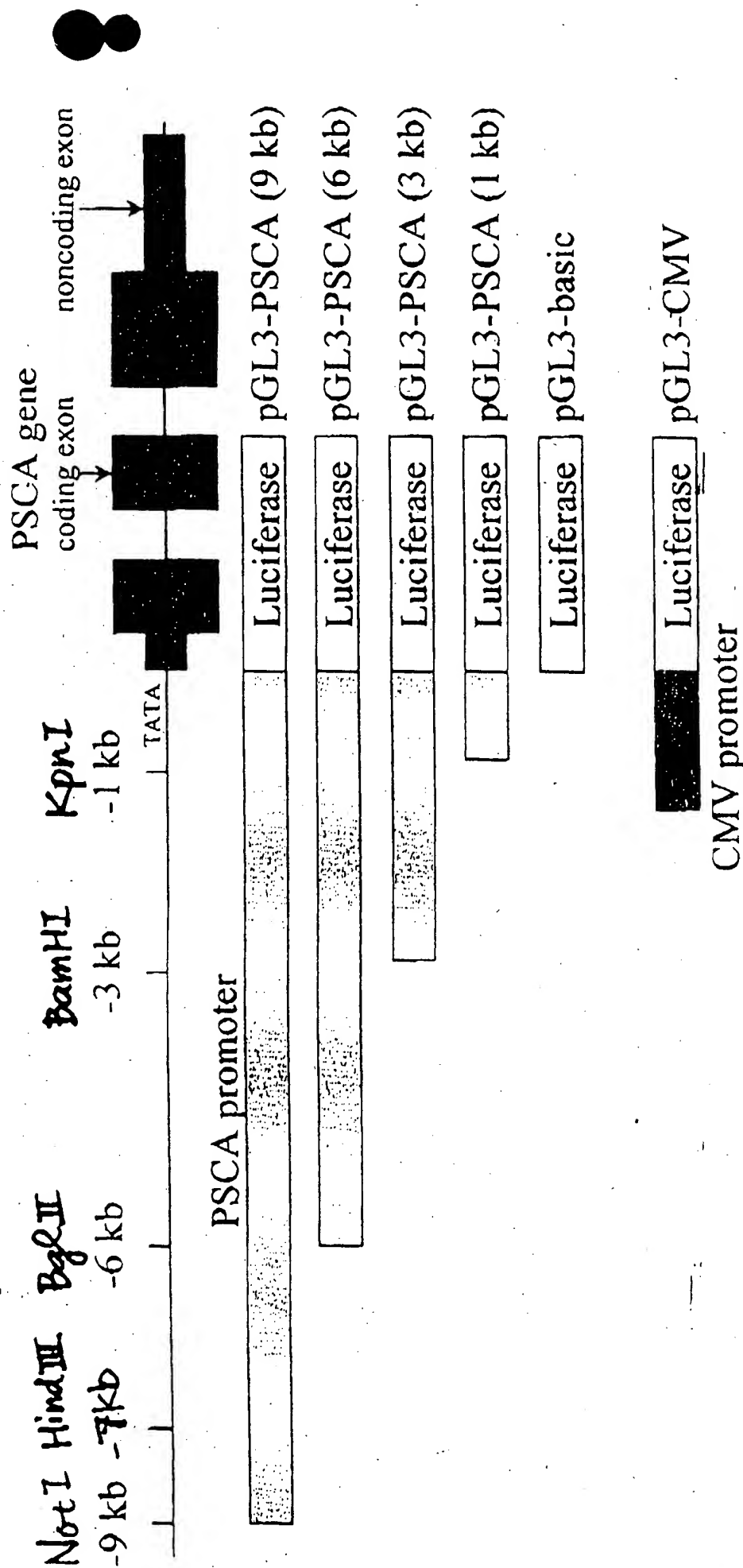


FIGURE 42



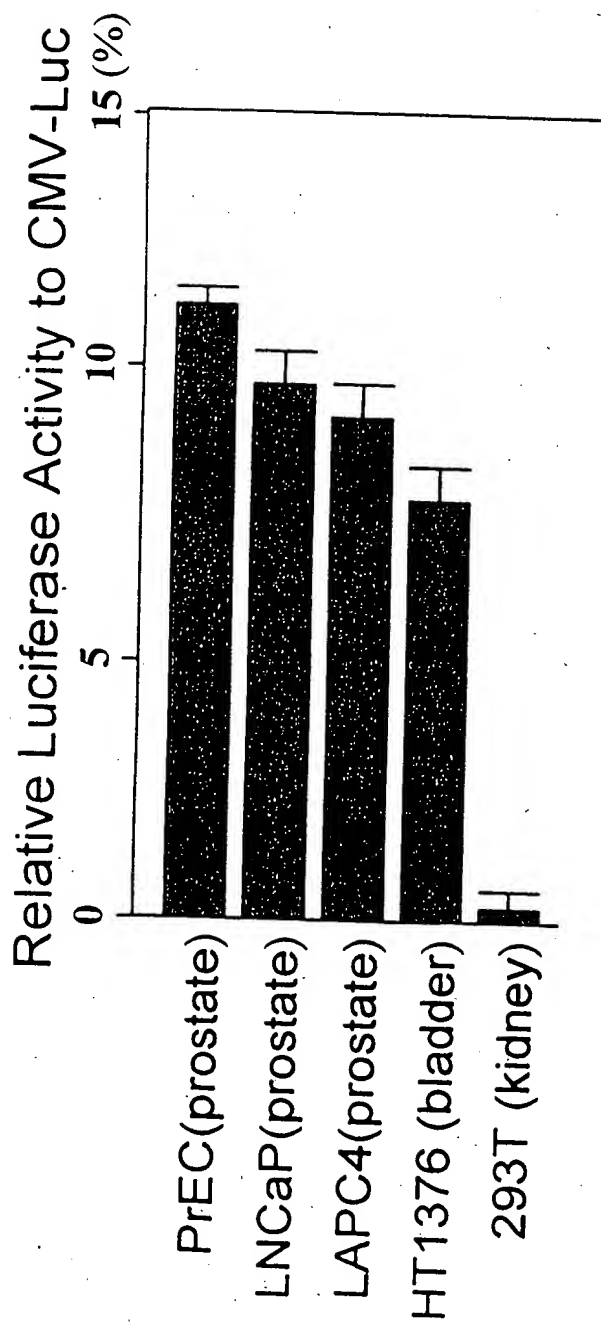


FIGURE 43

# Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

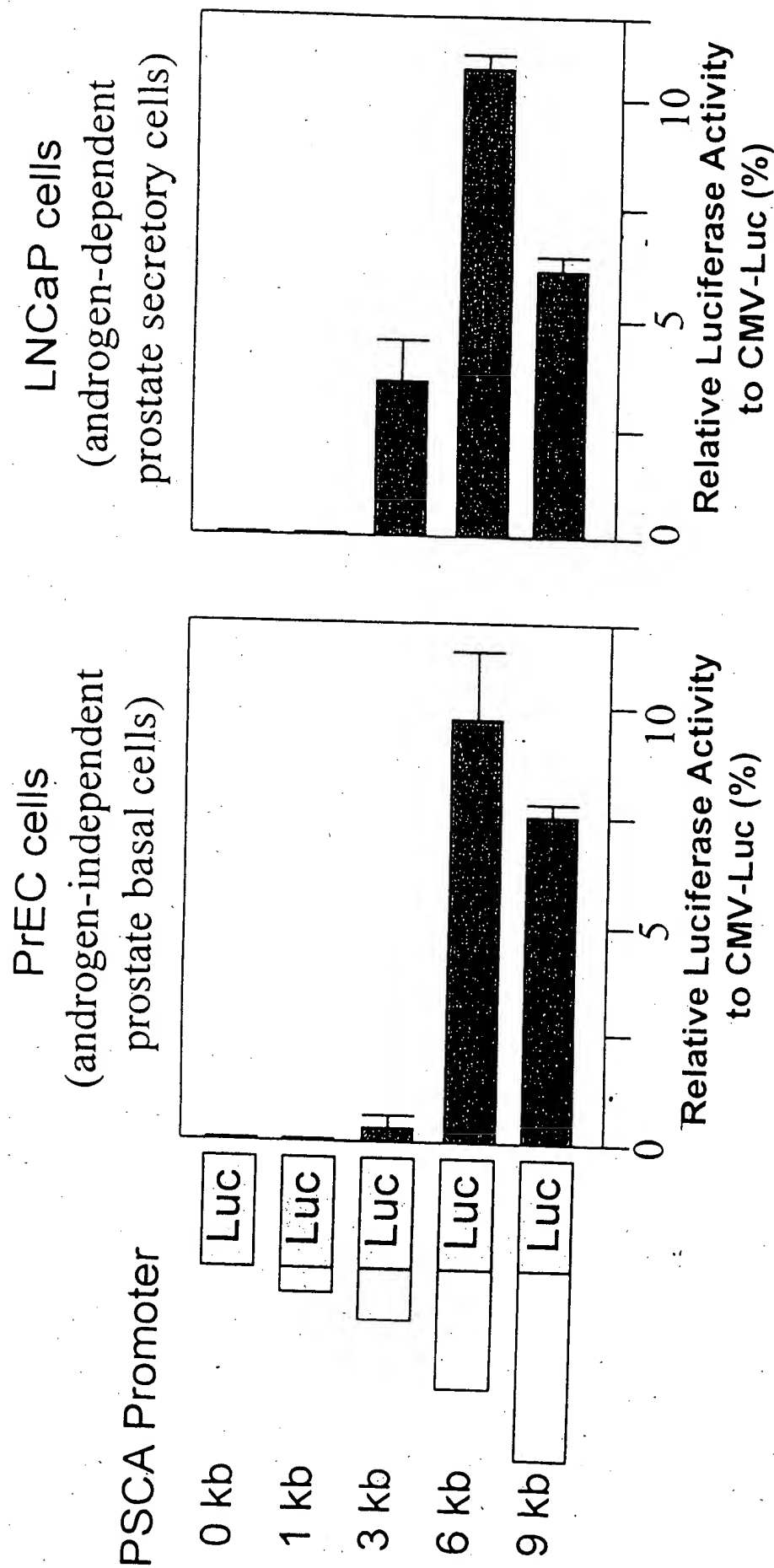


FIGURE 44

# Update of Transgenic Mouse Projects

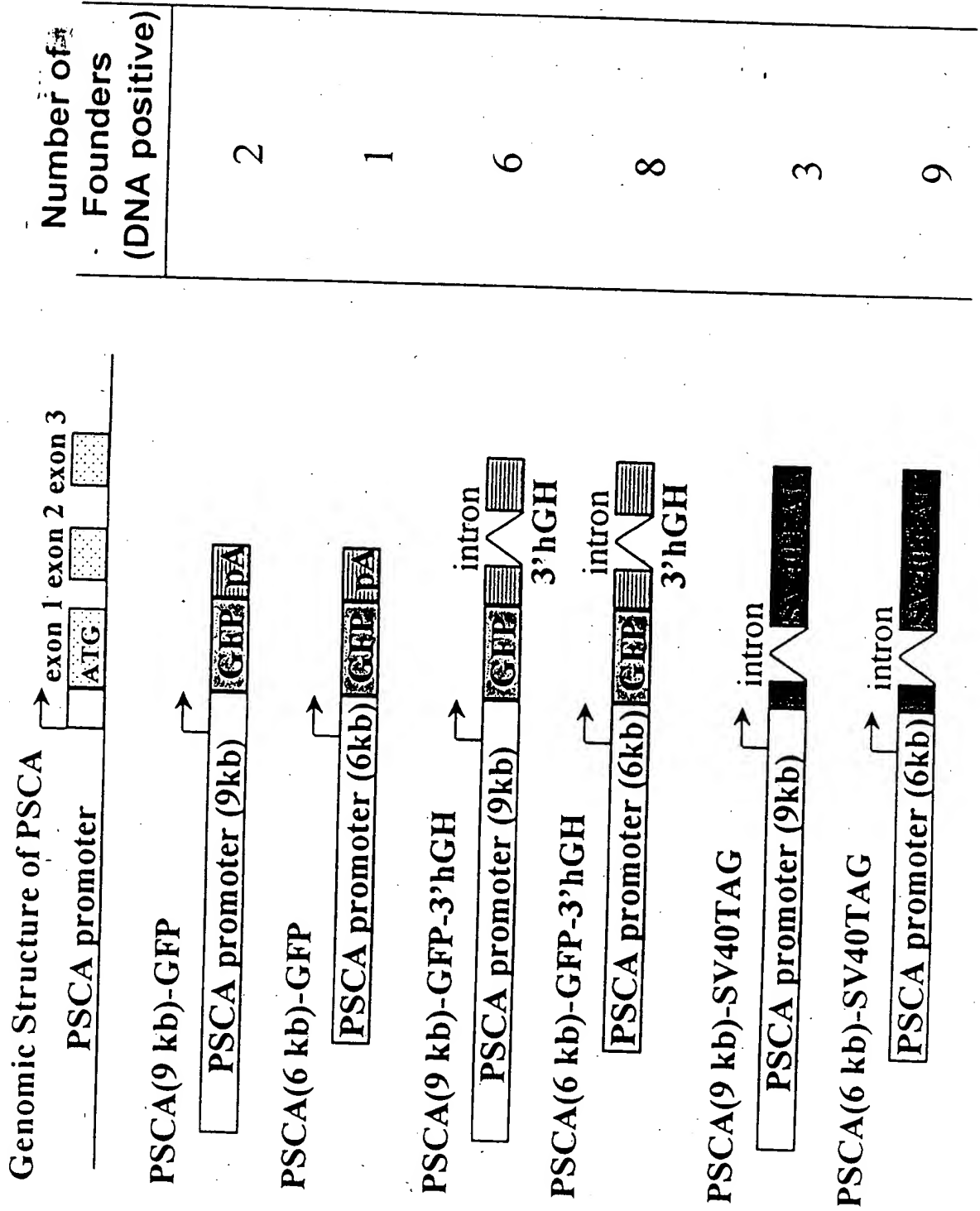


FIGURE 45

Negative tissues

Stomach

Small intestine

Colon

Seminal Vesicle

Urethra

Testis

Liver

Kidney

Lung

Brain

Heart

Skeletal muscle

Ovary

Uterus

Prostate

(A25-106-2)

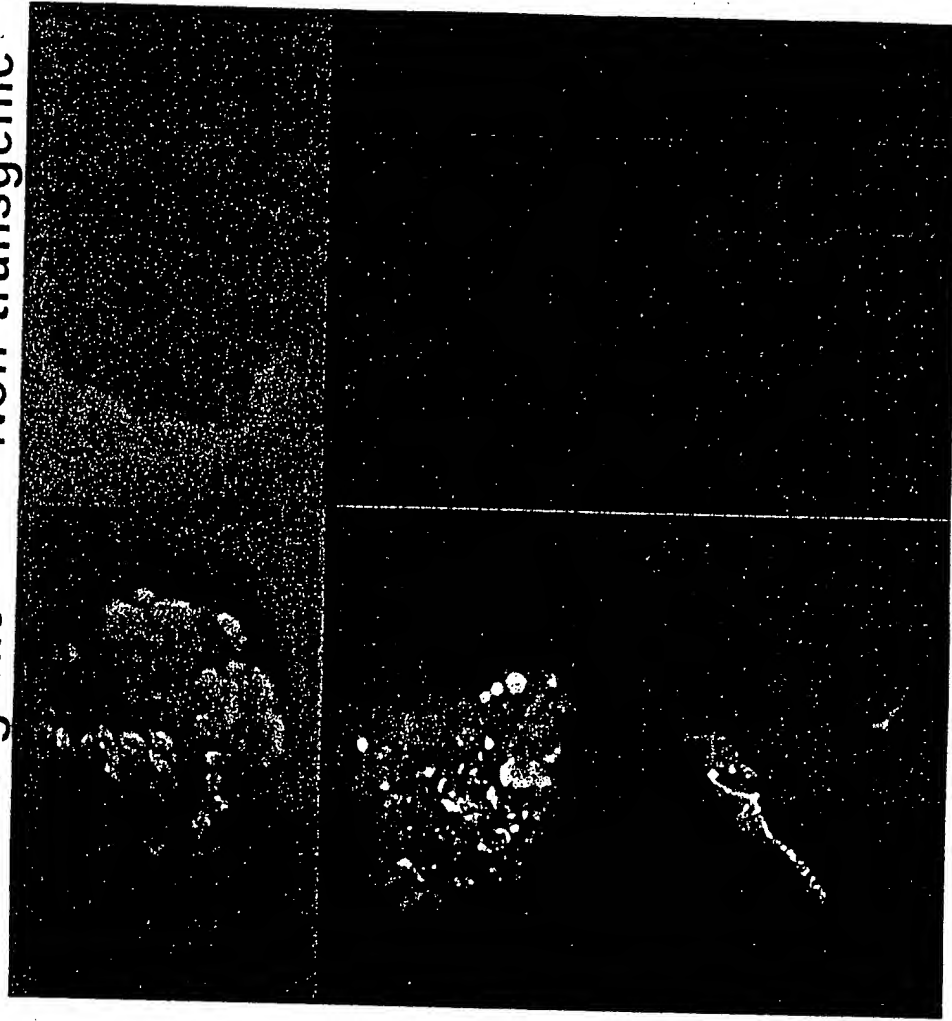
Bladder

(A25-104)

Skin

(A25-106-2)

Whole-mount green fluorescence image  
Transgenic Non-transgenic



HUMAN

Spleen  
Thymus  
Prostate  
Testis  
Ovary  
S. int.  
Colon  
PBL

Heart  
Brain  
Placenta  
Lung  
Liver  
Muscle  
Kidney  
Panc

hPSCA→

Northern Analysis

MOUSE

Ant. prostate  
Dorso/Lat. prostate  
Bladder prostate  
Seminal vesicle  
Urethra  
Testis  
Kidney  
Esophagus  
Cardiac stomach  
Body of stomach  
Pyloric stomach  
Duodenum  
Small intestine  
Colon  
Salivary gland  
Spleen  
Thymus  
Bone marrow  
Skeletal muscle  
Heart  
Brain  
Eye  
Lung  
Liver  
Skin

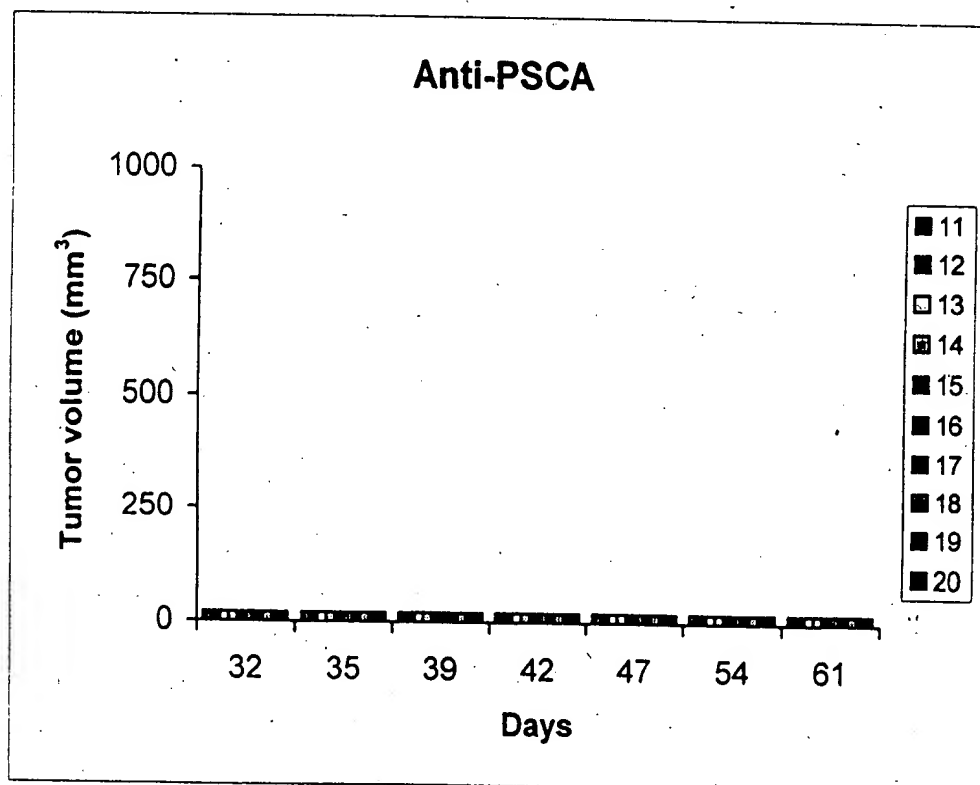
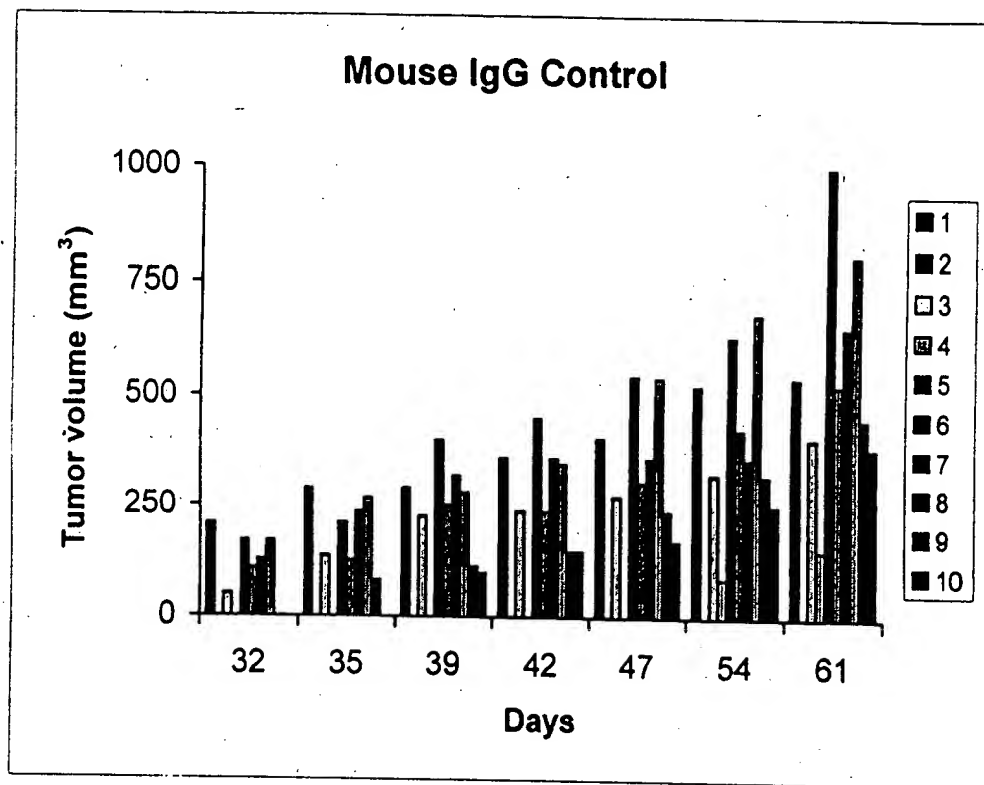
mPSCA→

mG3PDH→

RT-PCR

FIGURE 47

FIG. 48



**A**

**FIG. 49**

Epitope recognized (OD 450 nm)

<u>mAb</u>	<u>Isotype</u>	<u>F (18-98)</u>	<u>N (2-50)</u>	<u>M (46-109)</u>	<u>C (85-123)</u>
1G8	IgG1 k	1.485	0.004	1.273	0.003
2A2	IgG2a k	0.973	0.631	0.023	0.010
2H9	IgG1 k	1.069	1.026	0.002	0.001
3C5	IgG2a k	1.916	1.709	0.006	0.002
3E6	IgG3 k	1.609	0.036	1.133	2.118
3G3	IgG2a k	2.805	1.731	0.004	0.000
4A10	IgG2a k	1.053	0.493	0.000	0.001

**B**

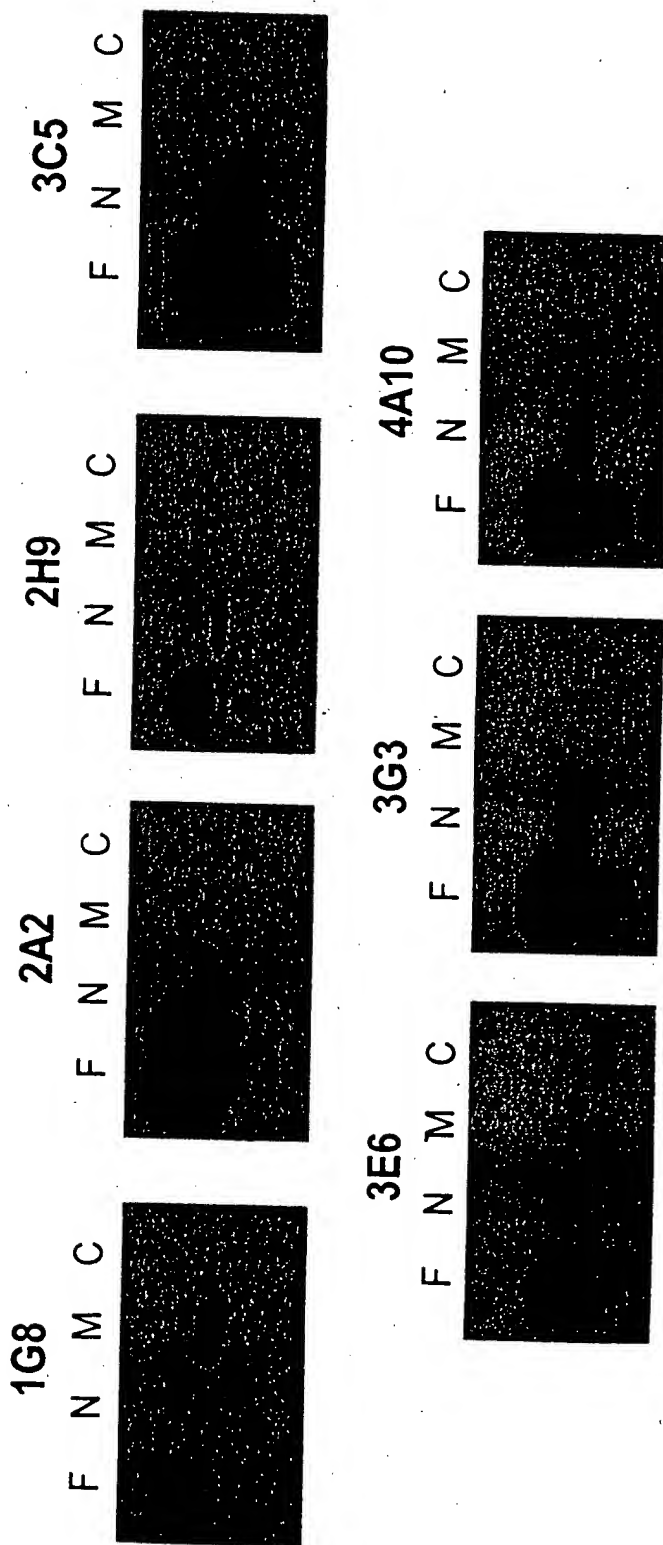
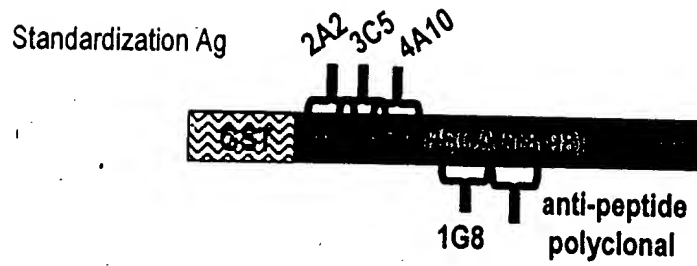
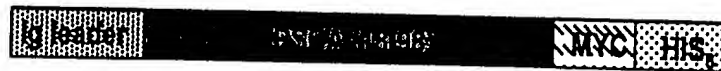


FIG. 50

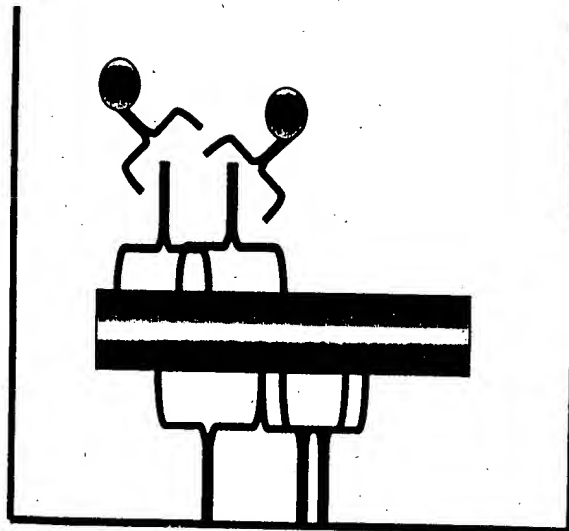
A



Engineered mammalian secreted form



B



Anti-IgG2a HRP

Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

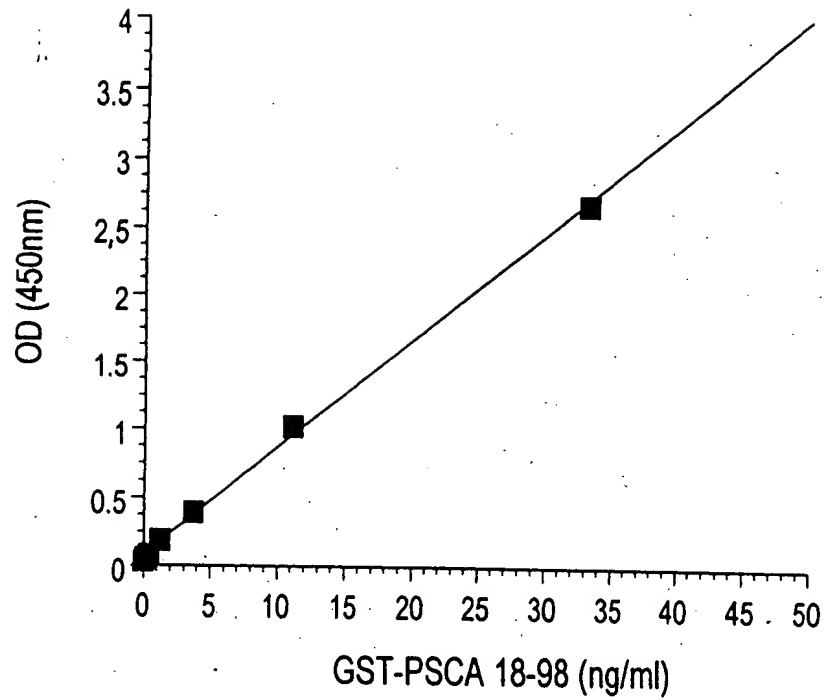
Affinity purified anti-peptide polyclonal  
+ mAb 1G8 (IgG1)

FIG. 50 EST 5860



FIG. 51

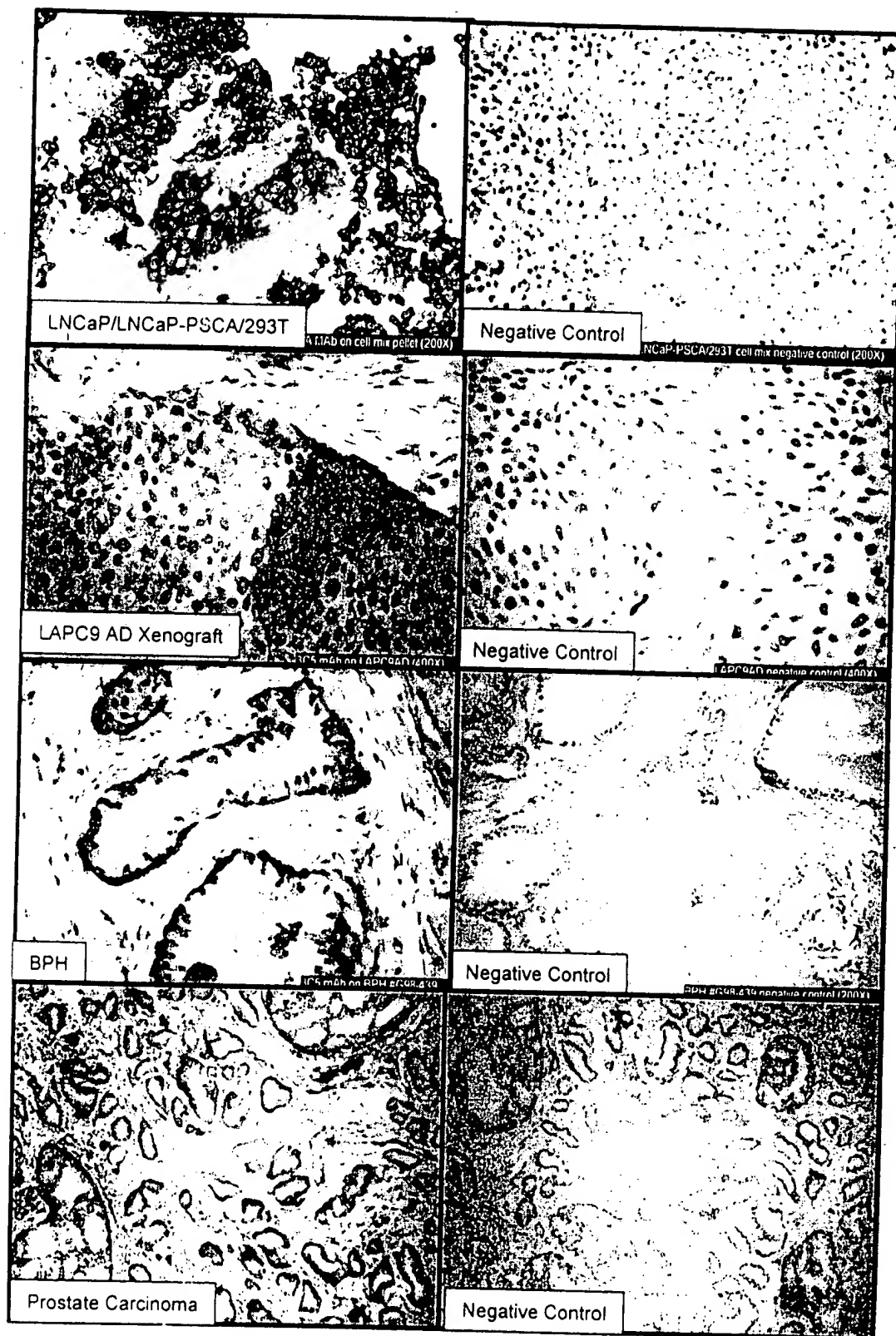
A



B

<u>Sample</u>	<u>OD+range (n=2)</u>	<u>ng/ml</u>
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52



0955153-05441

FIG. 53

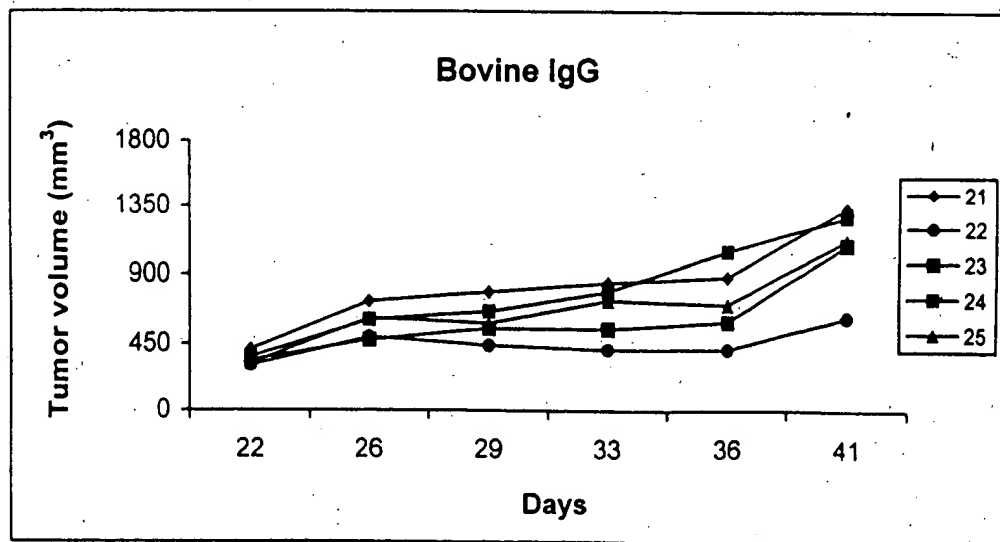
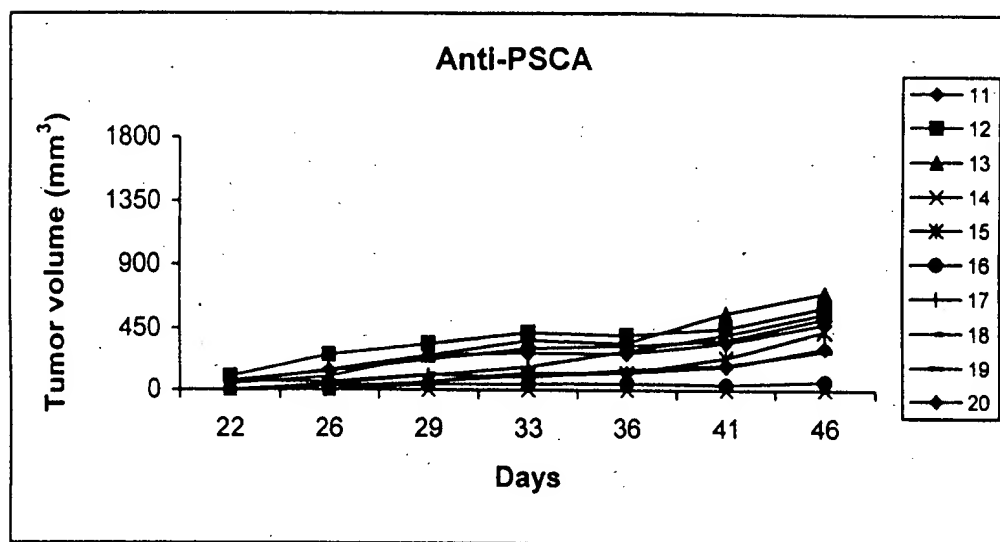
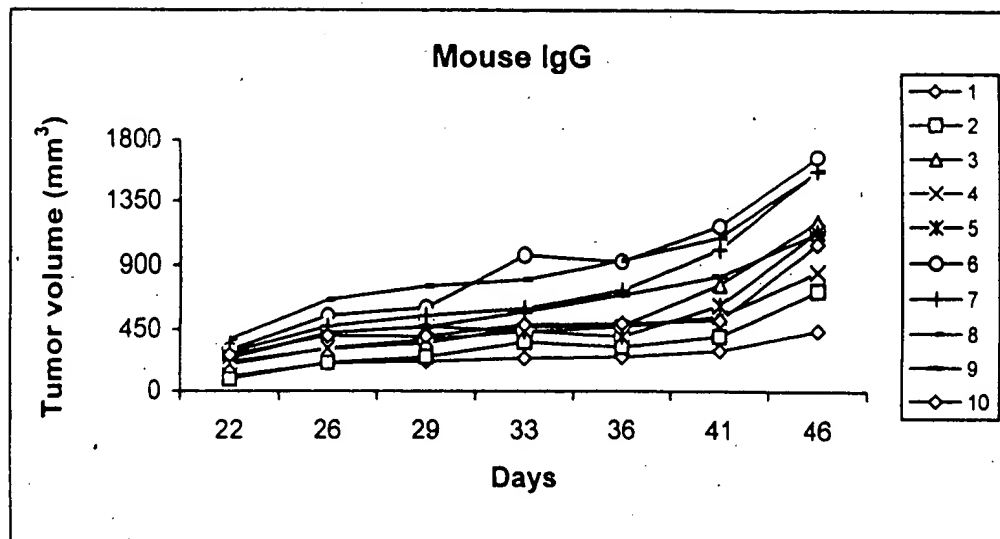


FIG. 53

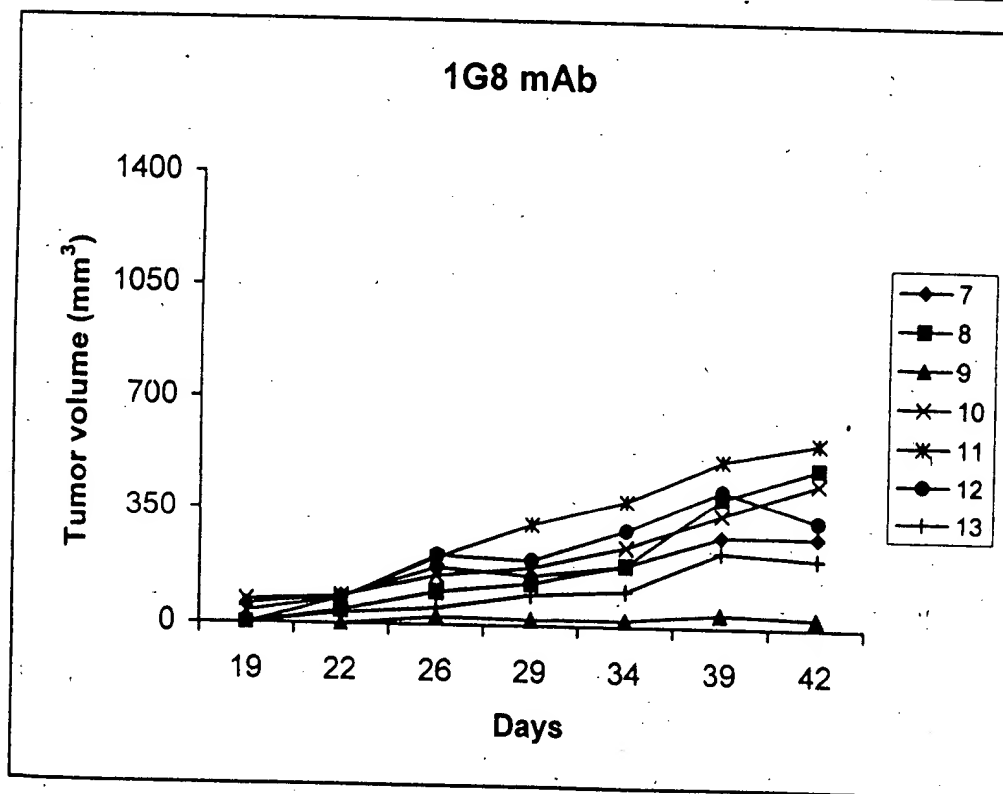
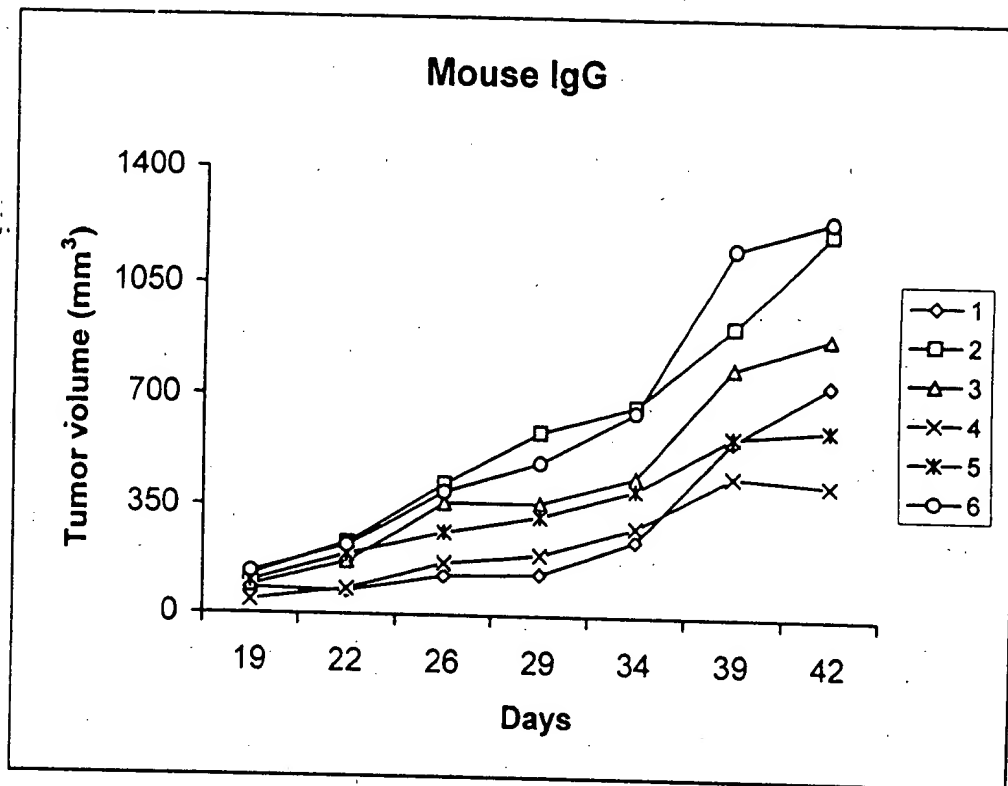


FIG. 55

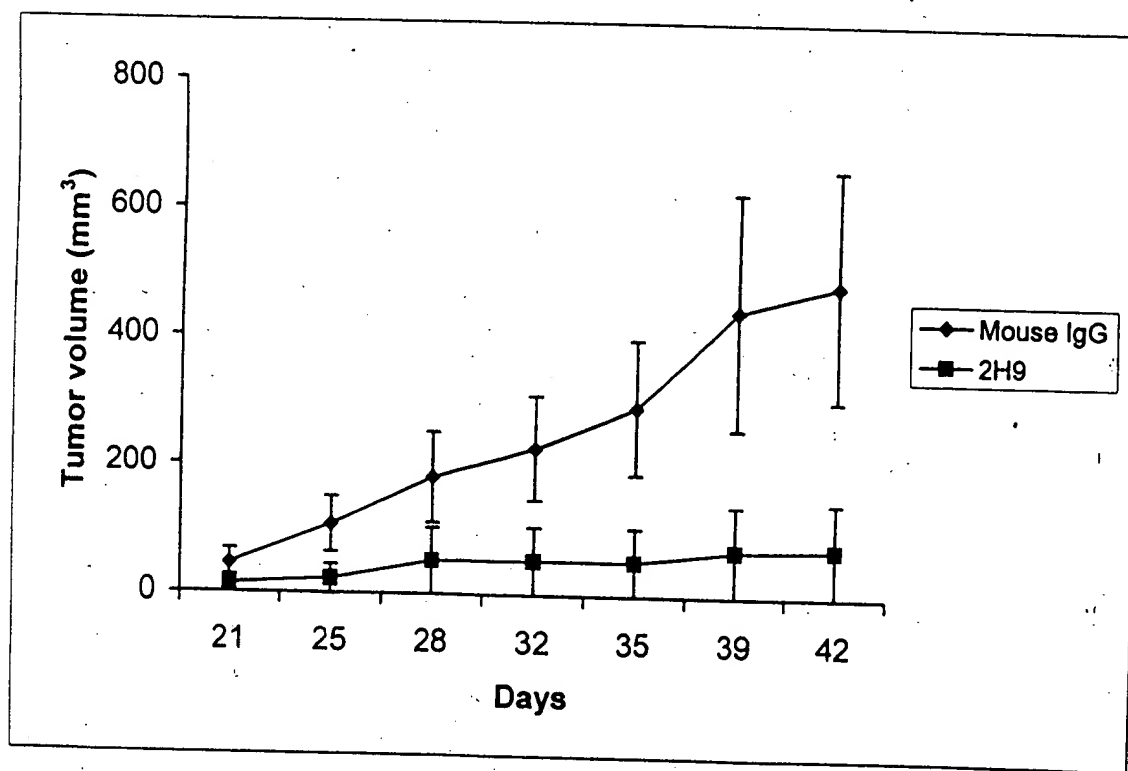
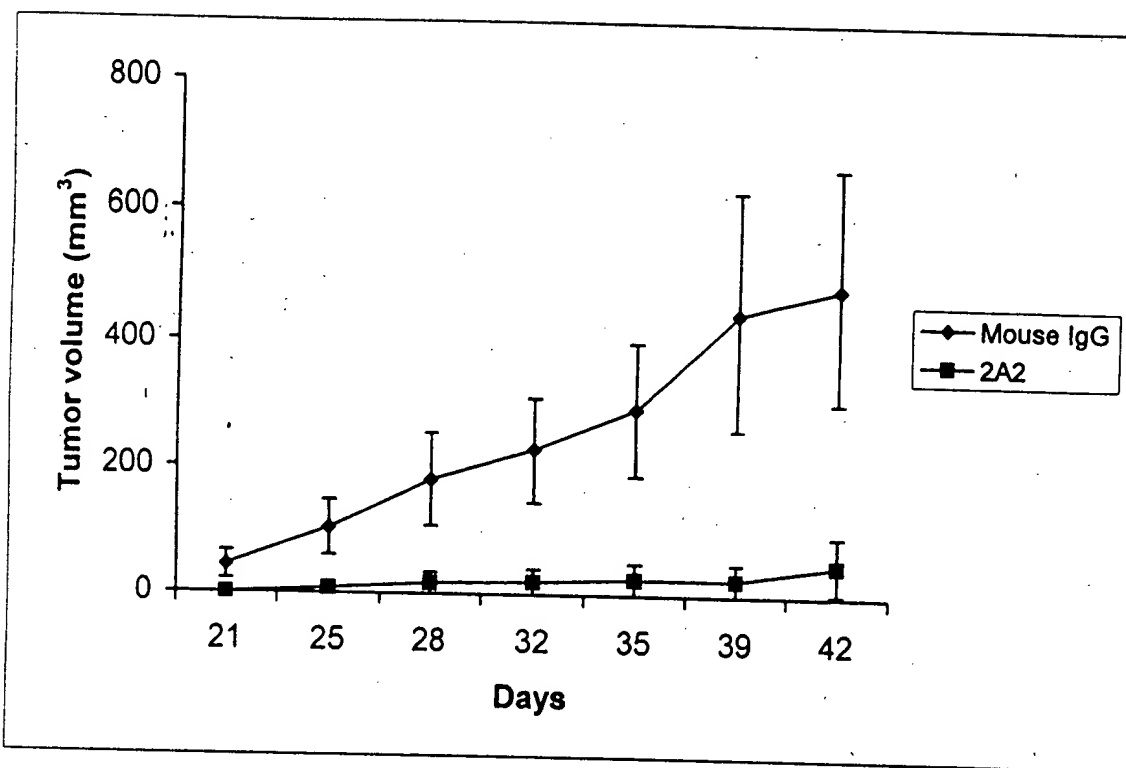


FIG. 56

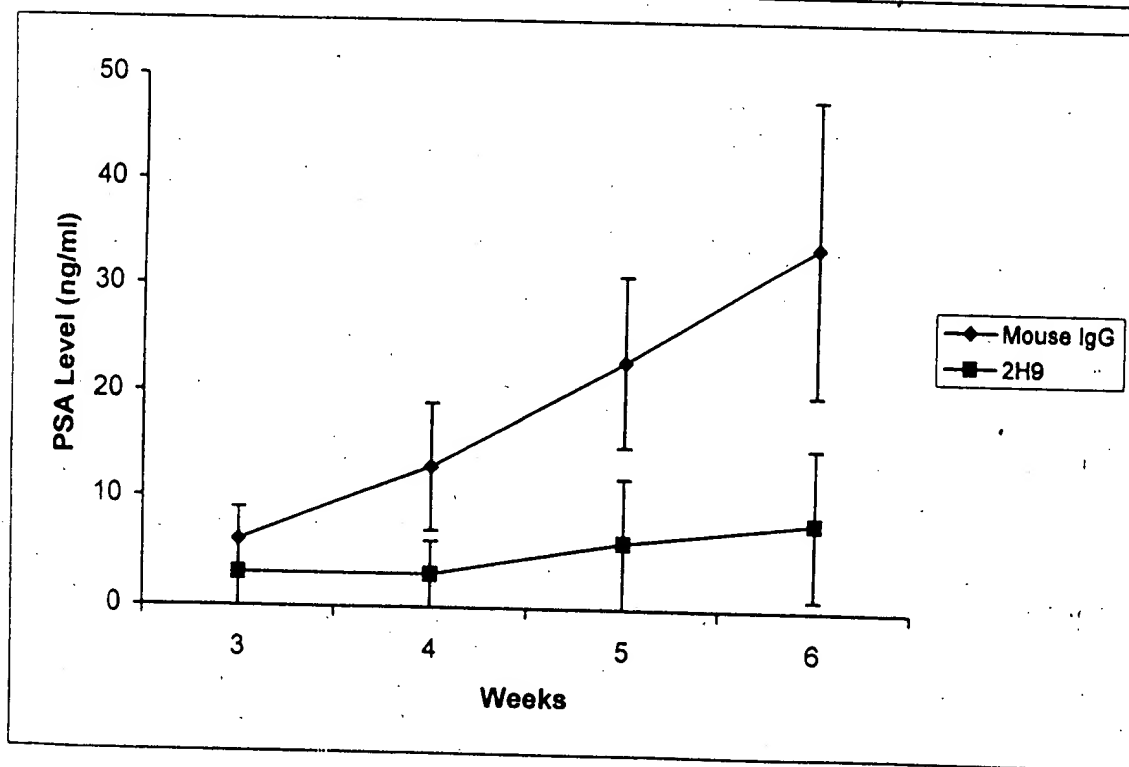
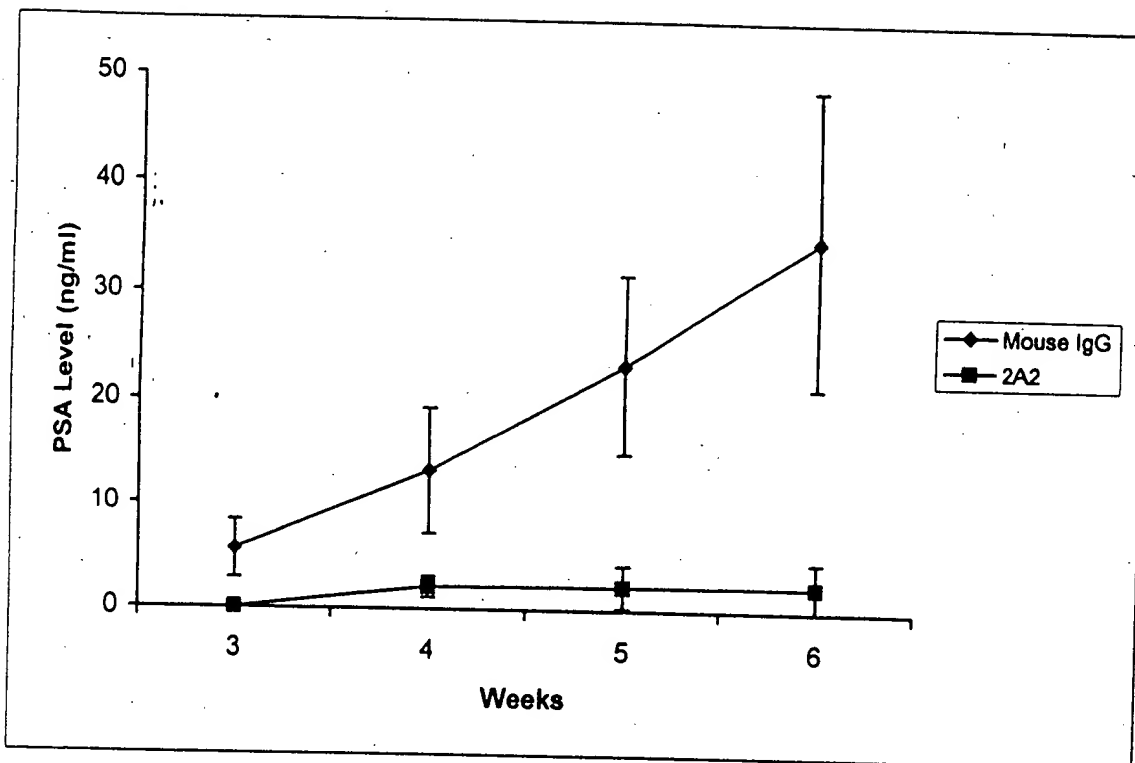


FIG. 57

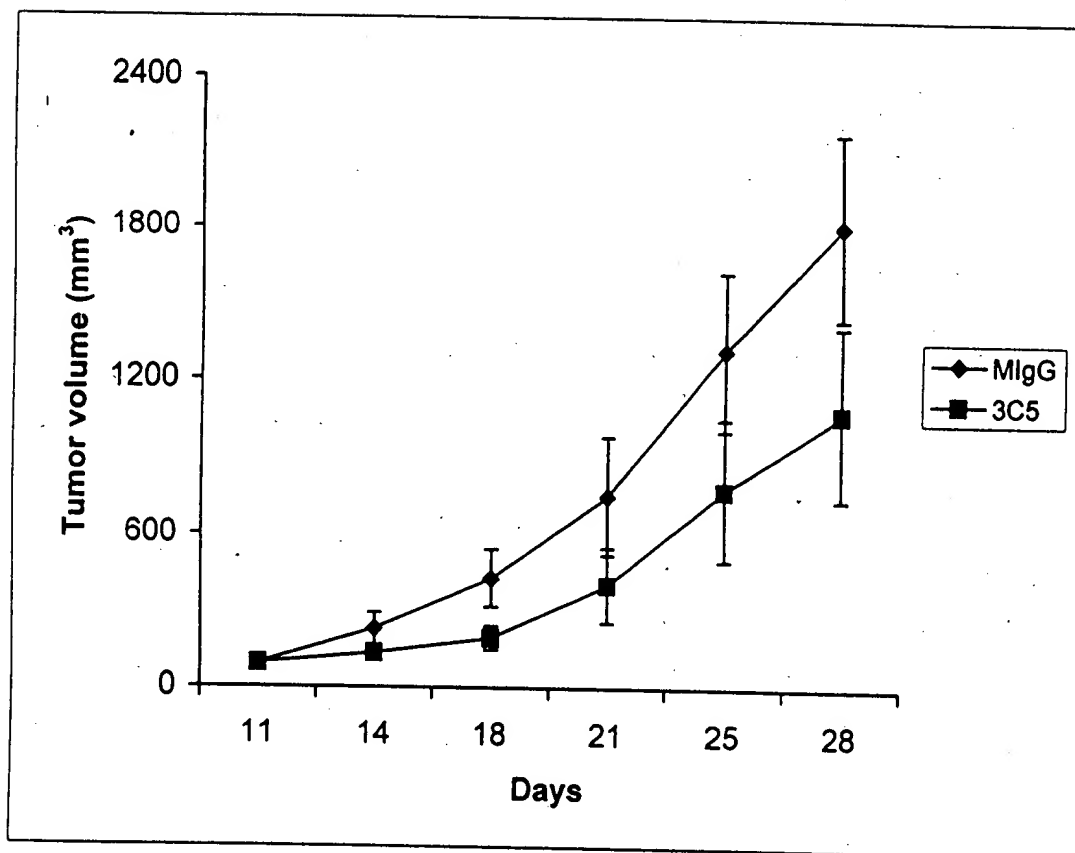


FIG. 58

TGCTTCTTCCTGATGGCAGTGGTTATAGGAGTCAATTCAGAGGTTTCAGCTGCAGCAGTCT 60  
C F F L M A V V I G V N S E V Q L Q Q S 20

GGGGCAGAACTTGTGAGGTCAGGGGCCTCAGTCAAGTTGTCCTGCACAGCTTCTGGCTTC 120  
G A E L V R S G A S V K L S C T A S G F 40

———— CDR1 ————  
AACATTAAAGACTACTATATACACTGGGTGAATCAGAGGCCTGACCAGGGCCTGGAGTGG 180  
N I K D Y Y I H W V N Q R P D Q G L E W 60

———— CDR2 ————  
ATTGGATGGATTGATCCTGAGAATGGTGACACTGAATTTGTCCCGAAGTTCCAGGGCAAG 240  
I G W I D P E N G D T E F V P K F O G K 80

GCCACTATGACTGCAGACATTTTCTCCAACACAGCCTACCTGCACCTCAGCAGCCTGACA 300  
A T M T A D I F S N T A Y L H L S S L T 100

———— CDR3 ————  
TCTGAAGACACTGCCGTCTATTACTGTAAAACGGGGGGTTTCTGGGGCCAAGGGACTCTG 360  
S E D T A V Y Y C K T G G F W G Q G T L 120

GTCACTGTCTCTGCAGCCAAAACGACACCCCCATCTGTCTATCCACTG  
V T V S A A K T T P P S V Y P L

095153 051404



FIG. 59

TTGGTAGCAACAGCCTCAGATGTCCACTCCCAGGTCCAAGTGCAGCAACCTGGGTCTGAA 60  
L V A T A S D V H S Q V Q L Q Q P G S E 20

CTGGTGAGGCCTGGAAGTTCAGTGAAGCTGTCCTGCAAGGCTTCTGGCTATACATTCTCC 120  
L V R P G T S V K L S C K A S G Y T F S 40  
CDR1

AGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGCCTTGAGTGGATTGGAAAT 180  
S Y W M H W V K Q R P G Q G L E W I G N 60

ATTGACCCTGGTAGTGGTTACACTAACTACGCTGAGAACCTCAAGACCAAGGCCACACTG 240  
I D P G S G Y T N Y A E N L K T K A T L 80  
CDR2

ACTGTAGACACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC 300  
T V D T S S S T A Y M Q L S S L T S E D 100

TCTGCAGTCTATTACTGTACAAGCCGATCTACTATGATTACGACGGGATTGCTTACTGG 360  
S A V Y Y C T S R S T M I T T G F A Y W 120  
CDR3

GGCCAAGGGACTCTGGTCACTGTCTCTGCAGCTACAACAACAGCCCCATCTGTCTATCCA 420  
G Q G T L V T V S A A T T T A P S V Y P 160

CTGGCC  
L A

0985153 051401

FIG. 60

AATGACTTCGGGTTGAGCTGGGTTTTTATTATTGTTCTTTTAAAGGGGTCCGGAGTGAA 60  
N D F G L S W V F I I V L L K G V R S E 20

GTGAGGCTTGAGGAGTCTGGAGGAGGCTGGGTGCAACCTGGAGGATCCATGAAACTCTCC 120  
V R L E E S G G G W V Q P G G S M K L S 40

TGTGTAGCCTCTGGATTTACTTTTCAGTAATTACTGGATGACTTGGGTCCGCCAGTCTCCA 180  
C V A S G F T F S N Y W M T W V R Q S P 60  
CDR1

GAGAAGGGGCTTGAGTGGGTTGCTGAAATTCGATTGAGATCTGAAAATTATGCAACACAT 240  
E K G L E W V A E I R L R S E N Y A T H 80  
CDR2

TATGCGGAGTCTGTGAAAGGGAAATTCACCATCTCAAGAGATGATTCCAGAAGTCGTCTC 300  
Y A E S V K G K F T I S R D D S R S R L 100

TACCTGCAAATGAACAACTTAAGACCTGAAGACAGTGGGAATTTATTACTGTACAGATGGT 360  
Y L Q M N N L R P E D S G I Y Y C T D G 120

CTGGGACGACCTAACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACA 420  
L G R P N W G Q G T L V T V S A A K T T 140  
CDR3

CCCCATCTGTCTATCCACTGGCCCCTTGTA  
P P S V Y P L A P C V

0965153-051401

FIG. 61

CDR1 Comparisons

1G8	1gG <sub>1k</sub>	Middle	G	F	N	I	K	D	Y	Y	I	H
2H9	1gG <sub>1k</sub>	N-Term.	G	F	T	F	S	N	Y	W	M	T
4A10	1gG <sub>2ak</sub>	N-Term.	G	Y	T	F	S	S	Y	W	M	H

CDR2 Comparisons

1G8	1gG <sub>1k</sub>	W	I	D	P	E	N	G	D	T	E	F	V	P	K	F	Q	G		
2H9	1gG <sub>1k</sub>	E	I	R	L	R	S	E	N	Y	A	T	H	Y	A	E	S	V	K	G
4A10	1gG <sub>2ak</sub>	N	I	D	P	G	S	G	Y	T	N	Y	A	E	N	L	K	T		

CDR3 Comparisons

1G8	1gG <sub>1k</sub>	G	G	F													
2H9	1gG <sub>1k</sub>	L	G	R	P	N											
4A10	1gG <sub>2ak</sub>	R	S	T	M	I	T	T	G	F	A	Y					

0055153-05401

FIG. 62

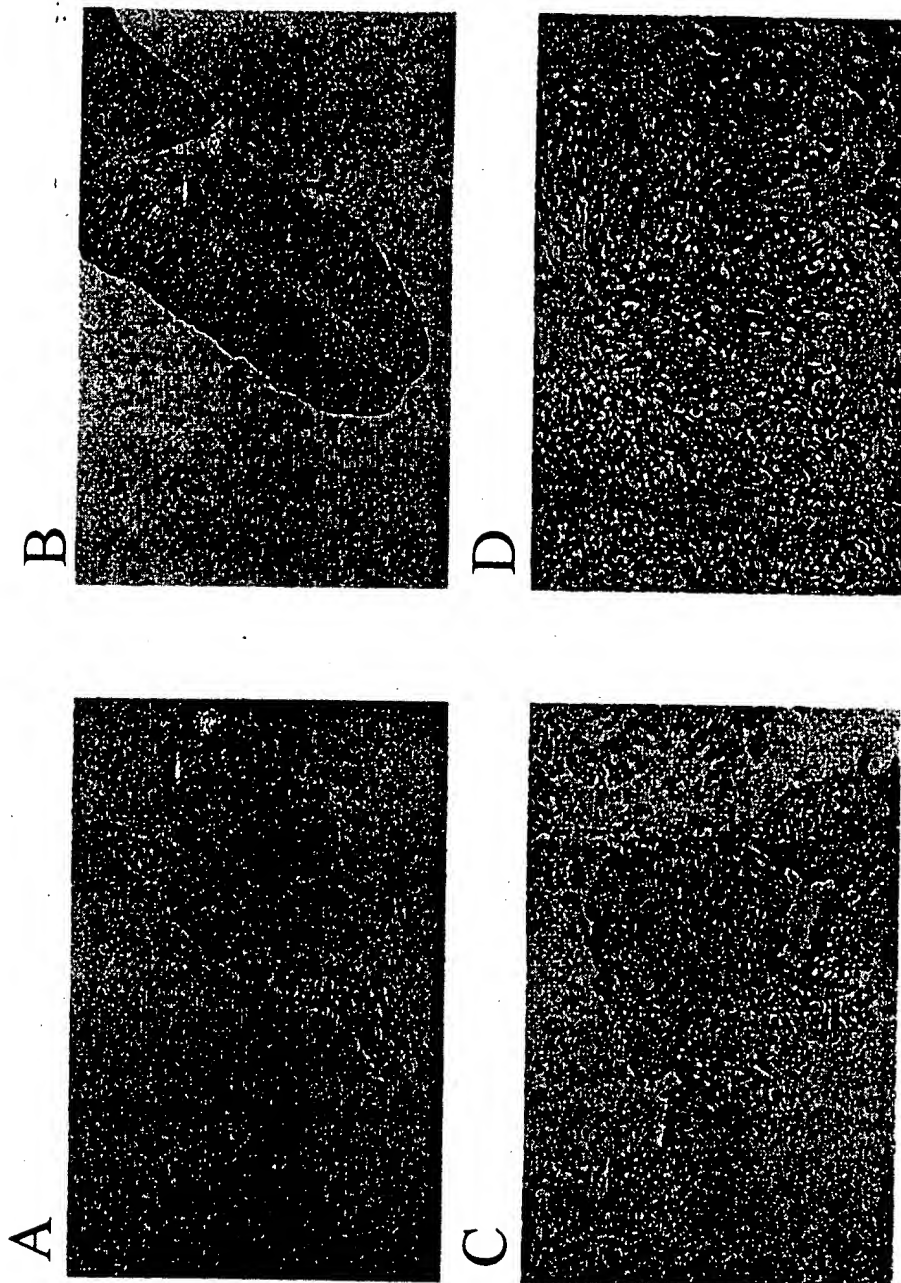


FIG. 63

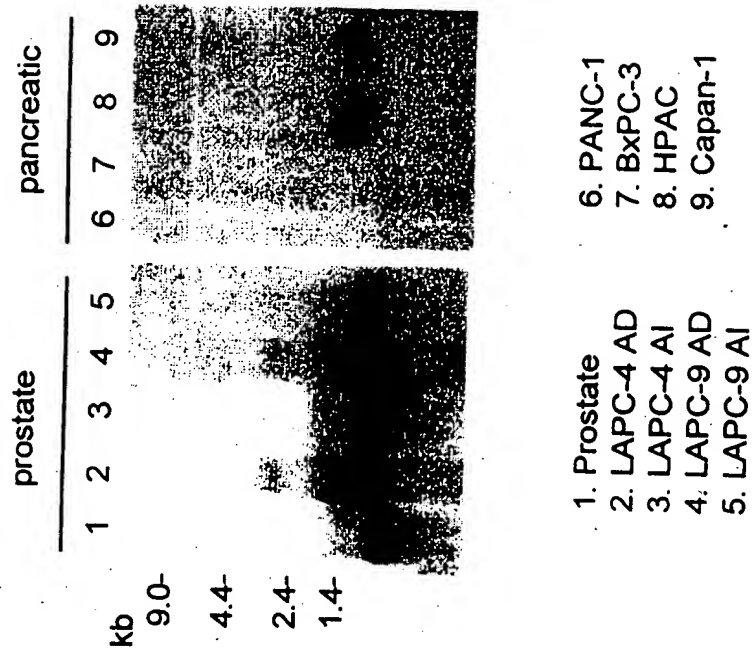
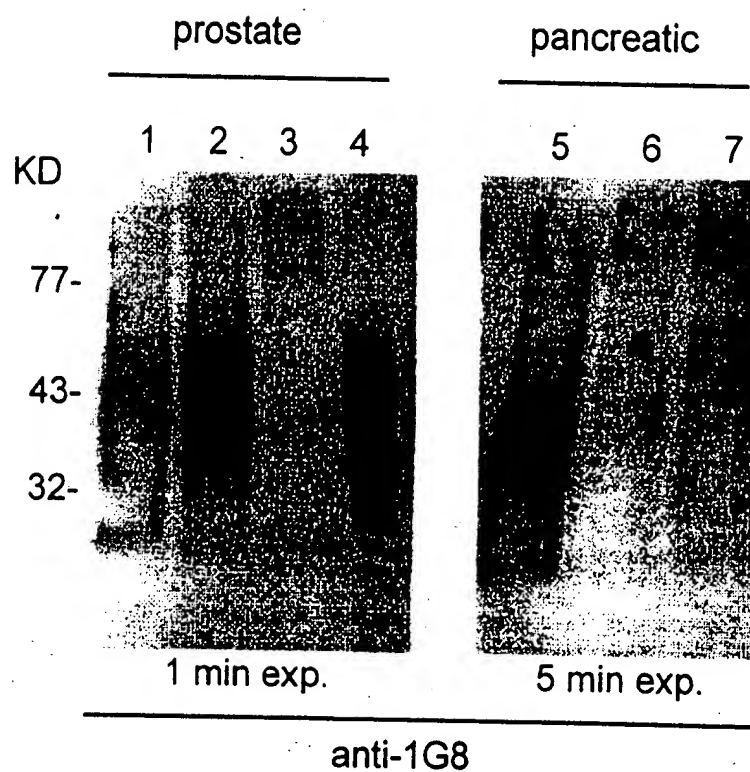


FIG. 64



- 1. LAPC-4 AD
- 2. LAPC-9 AI
- 3. LNCaP
- 4. LNCaP-PSCA
- 5. HPAC
- 6. Capan-1
- 7. ASPC-1

0055153-05401

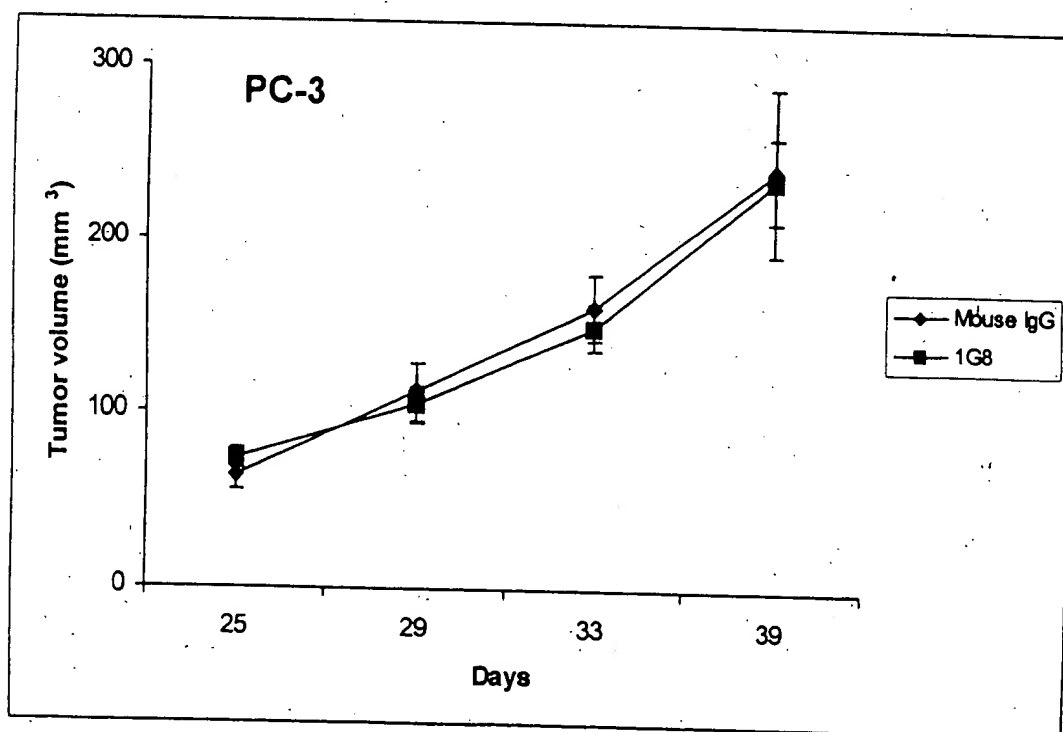
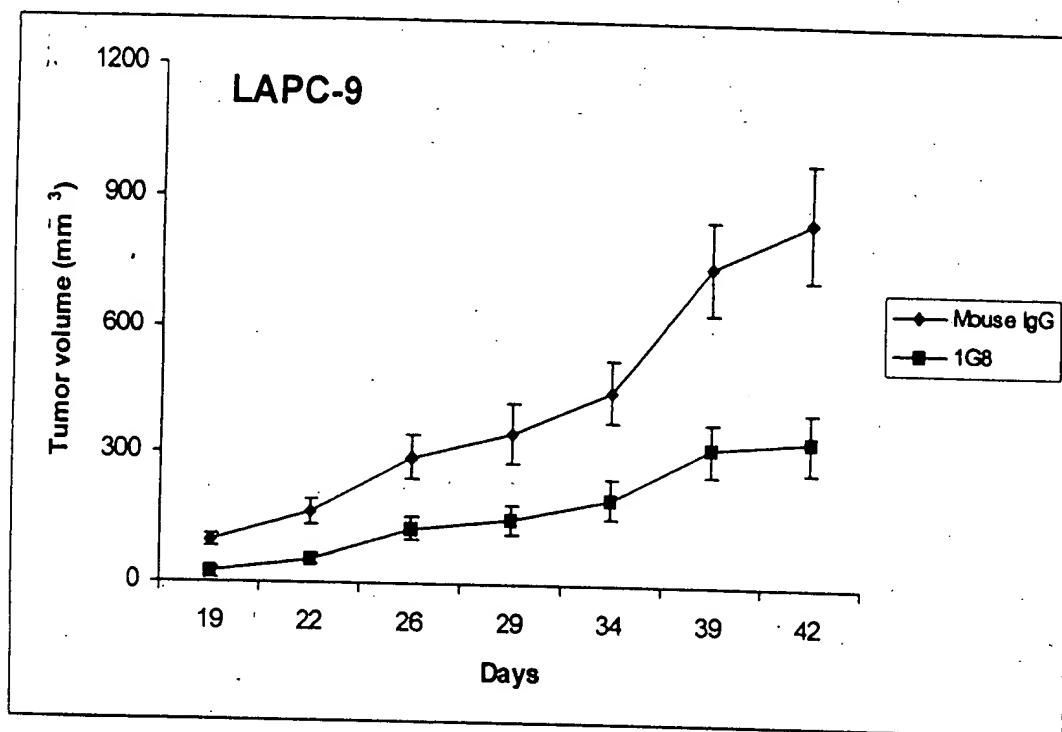
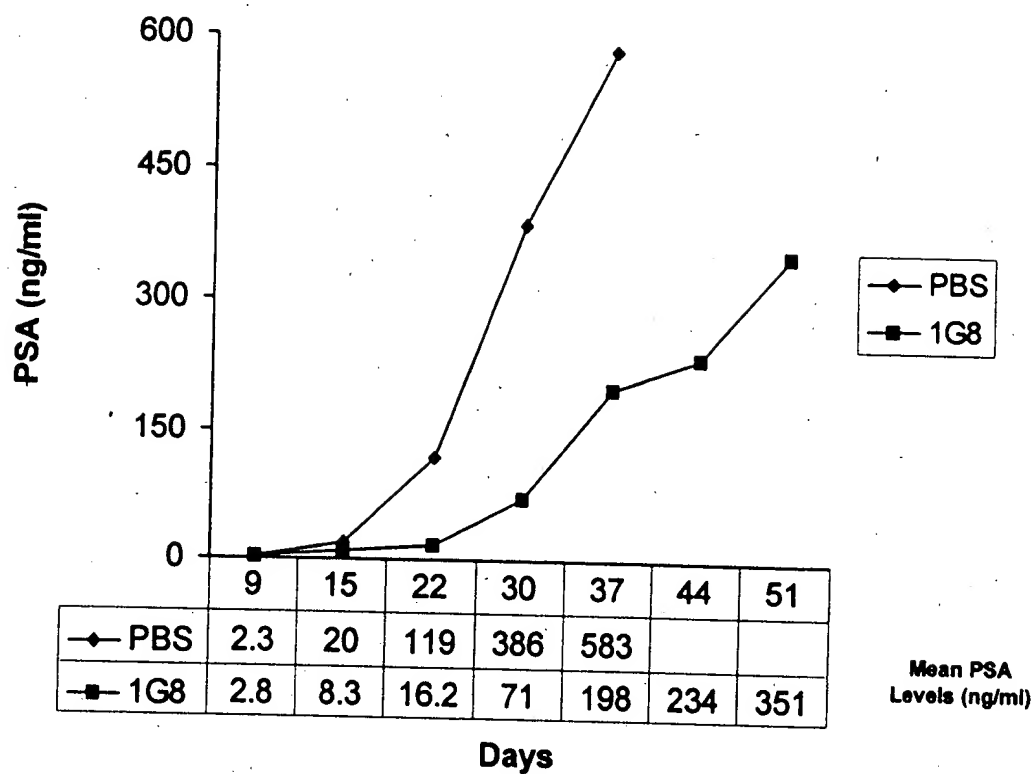


FIGURE 65

A)



B)

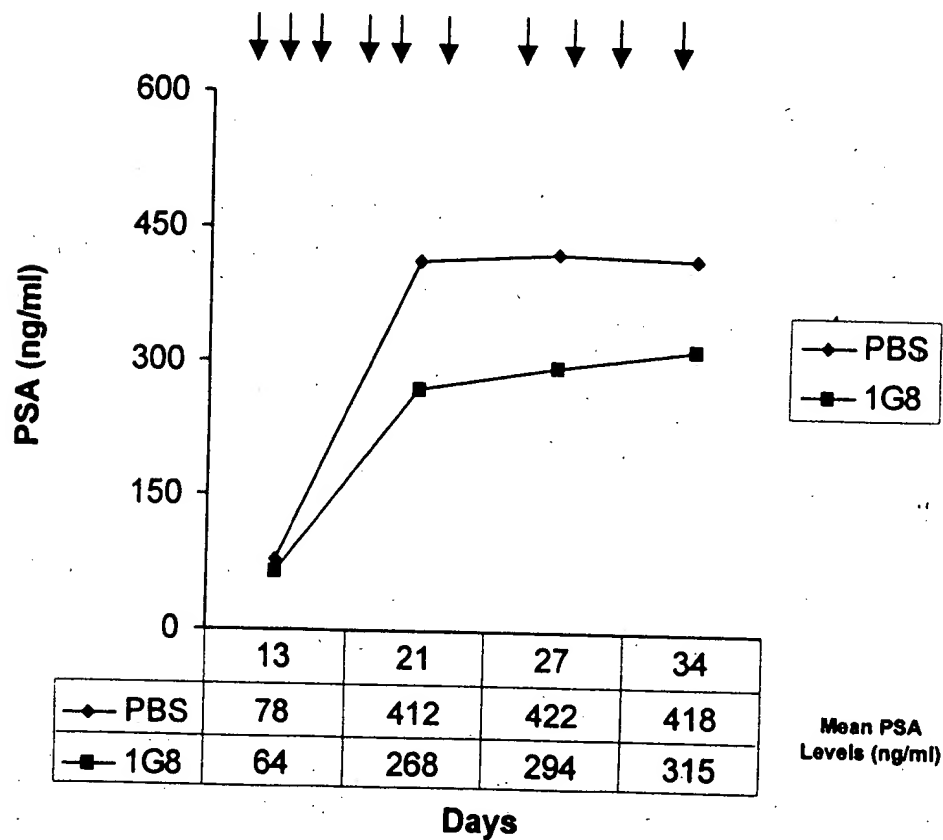
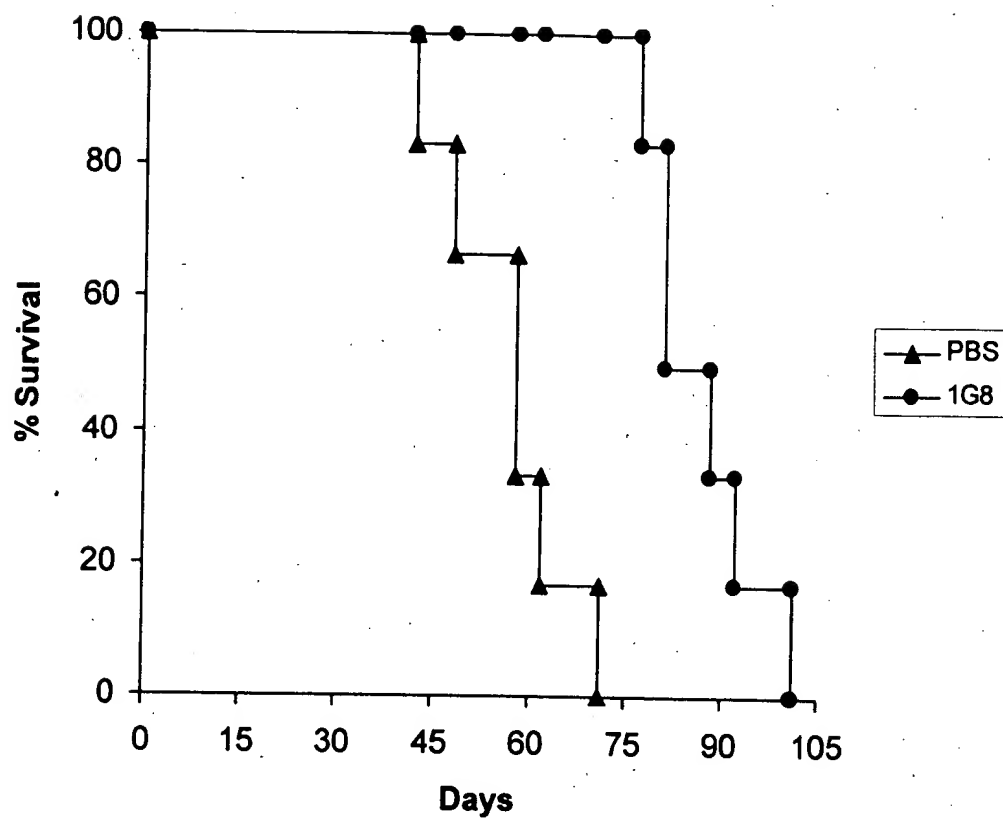


Figure 66



A)



B)

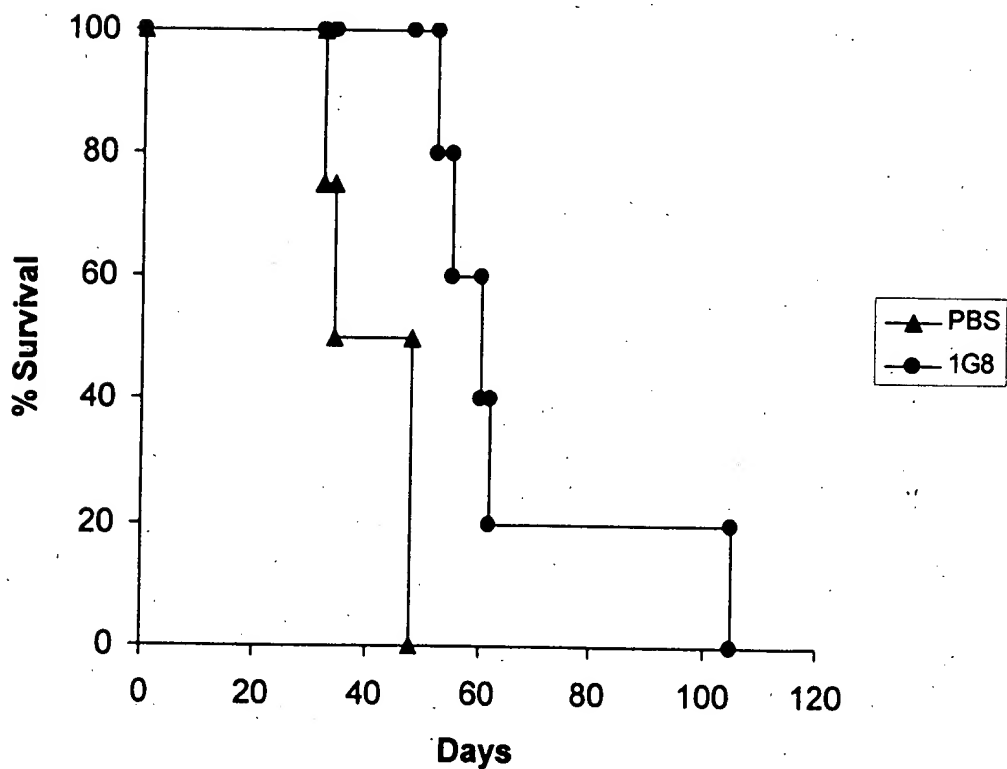
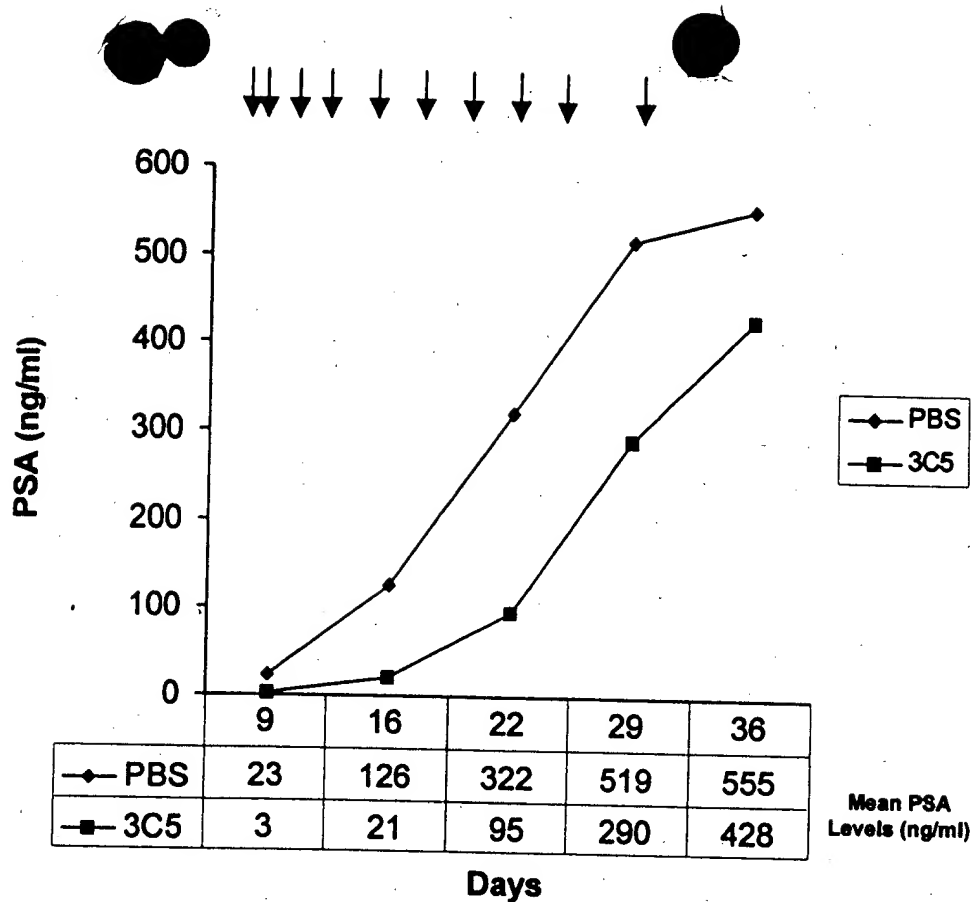


Figure 67

A)



B)

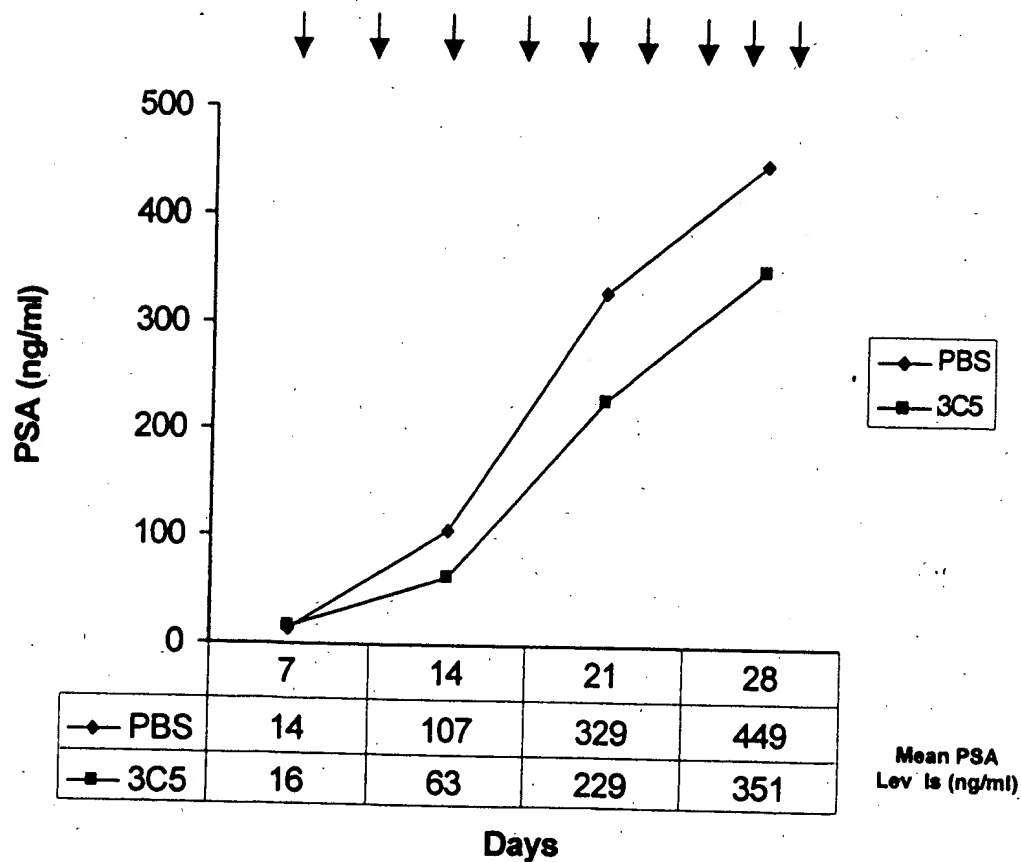
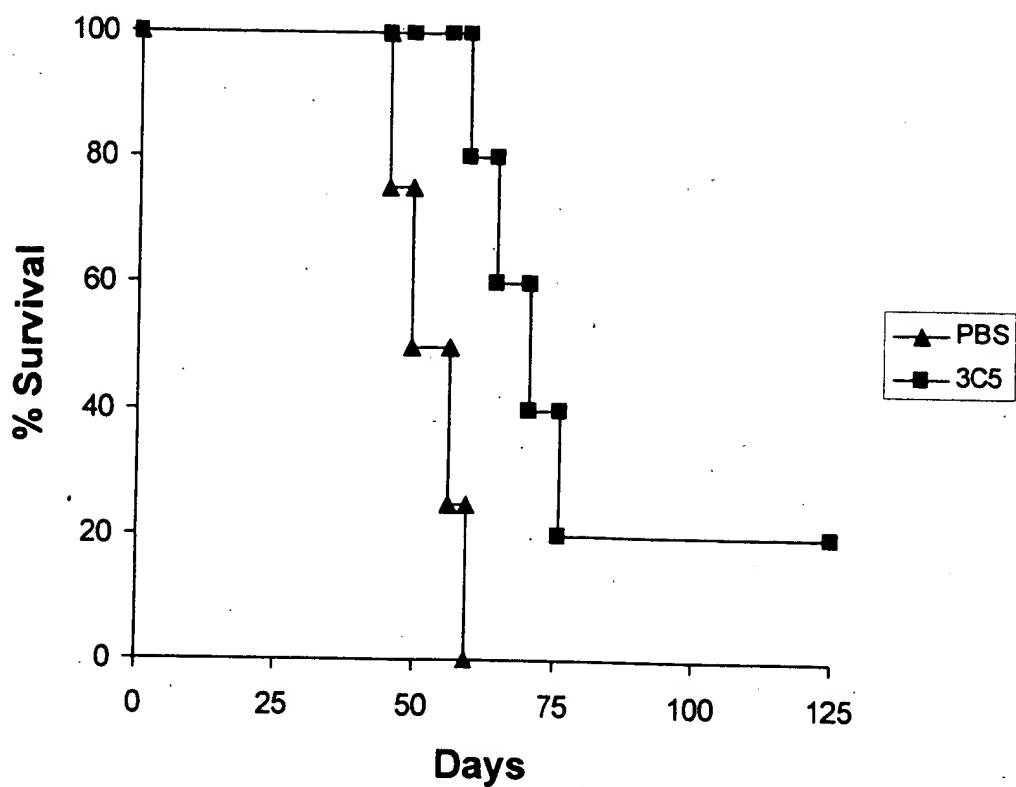


Figure 68

A)



B)

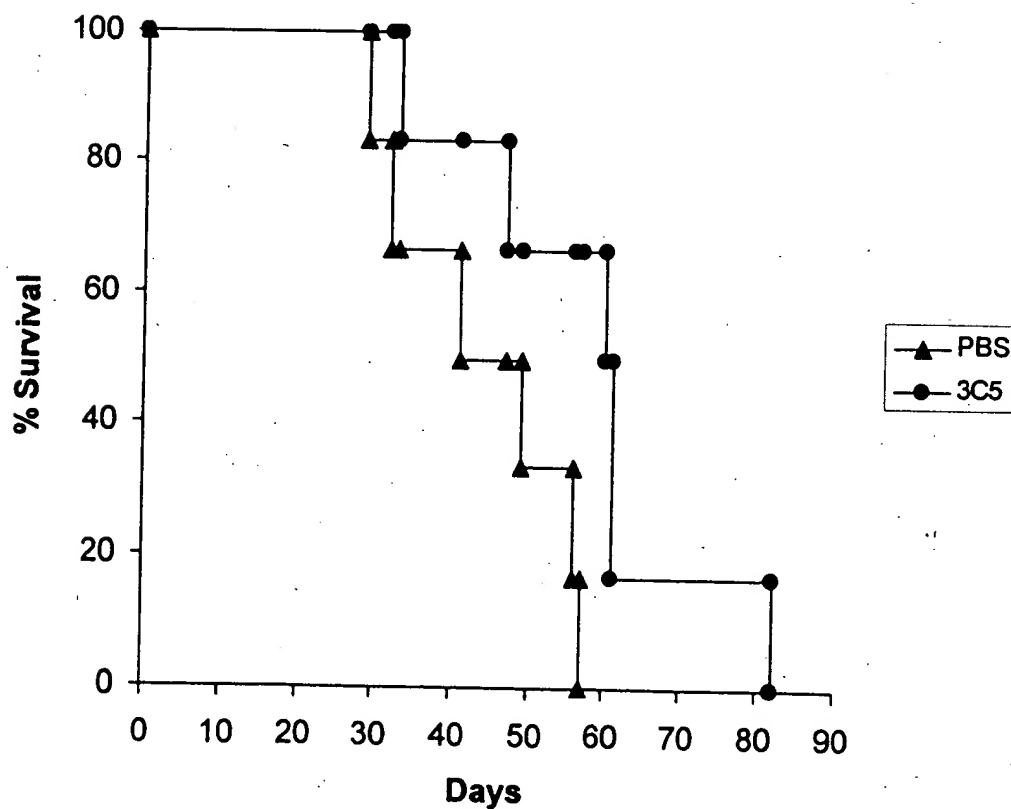


Figure 69

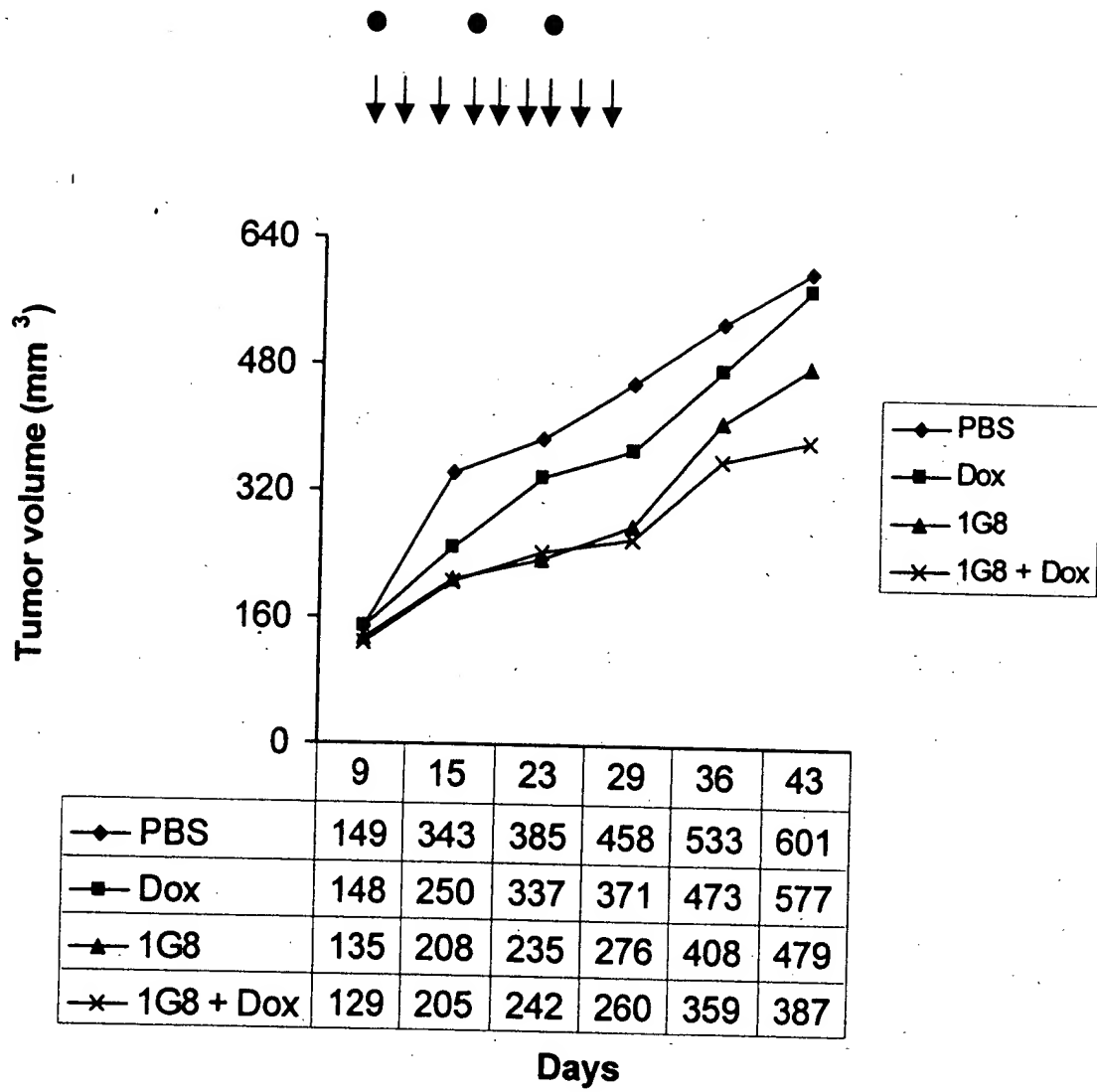
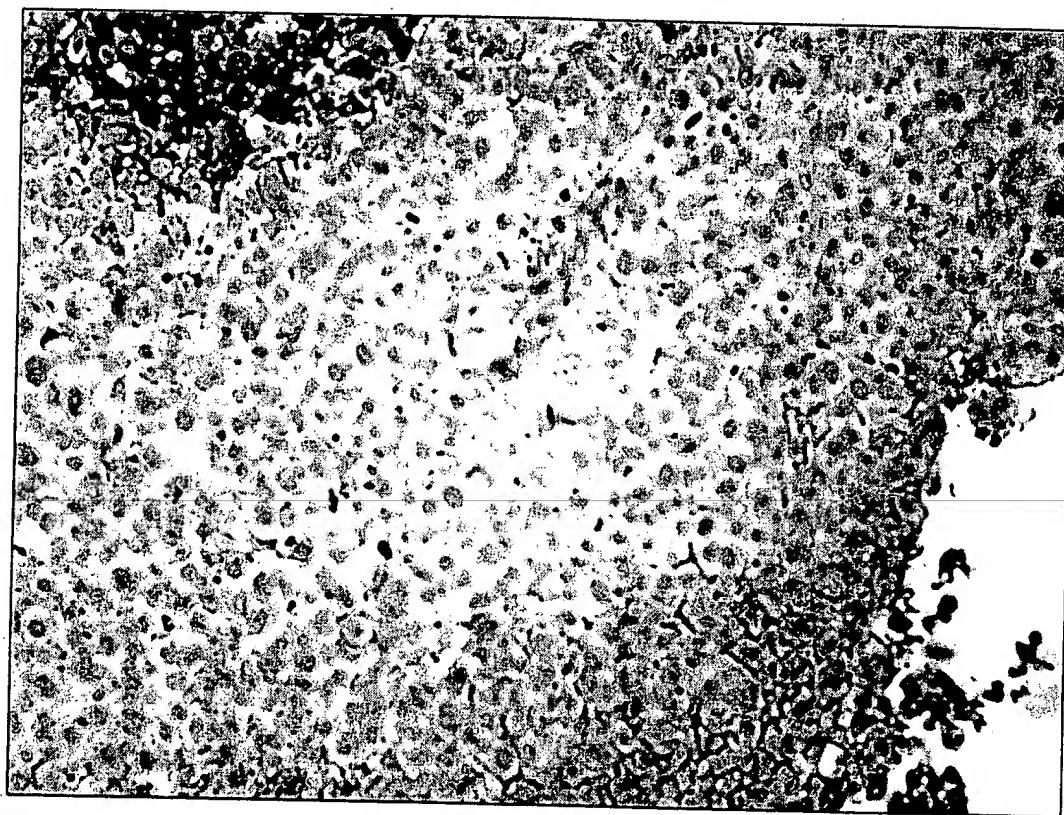


Figure 70

# PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue

3C5 Treated



mlgG Treated

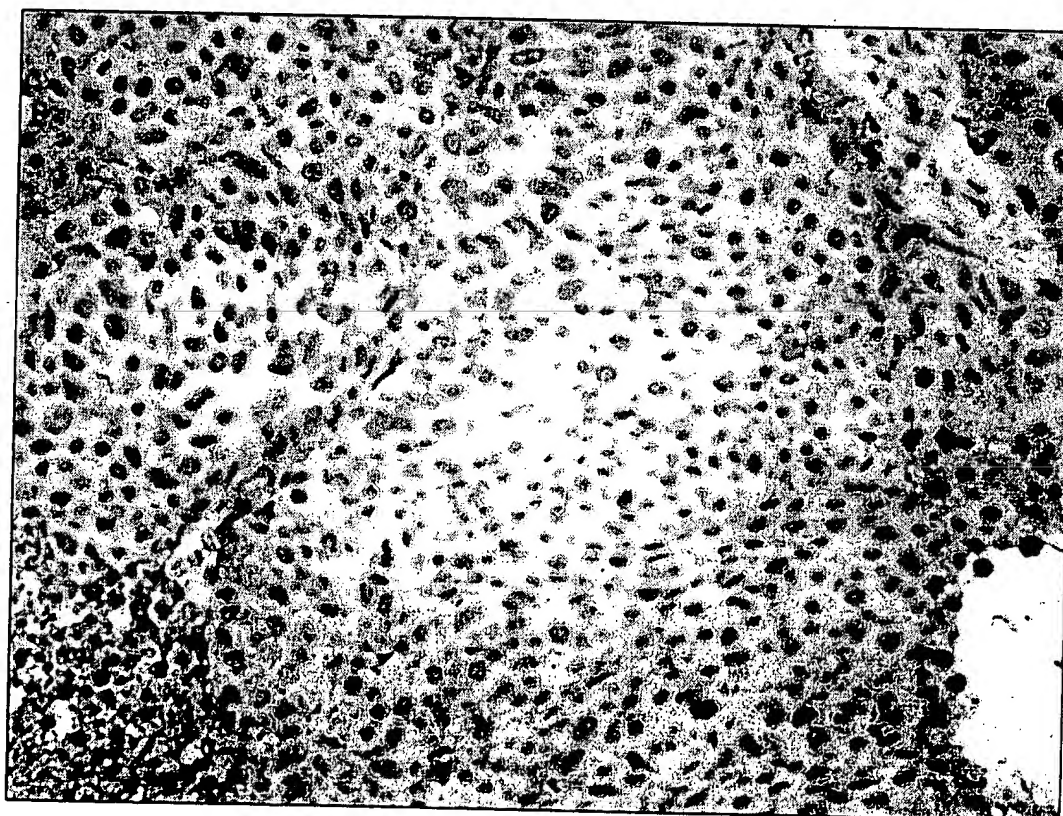
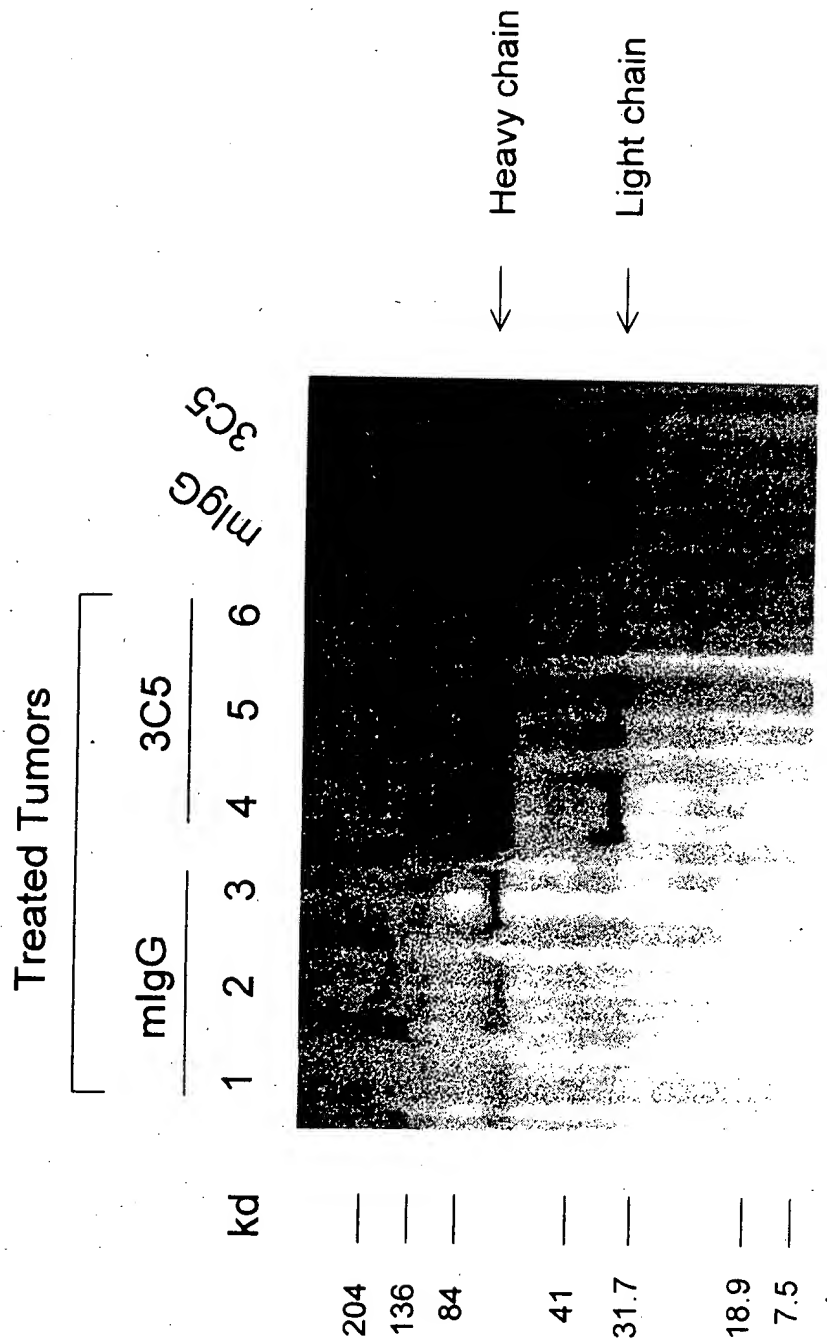


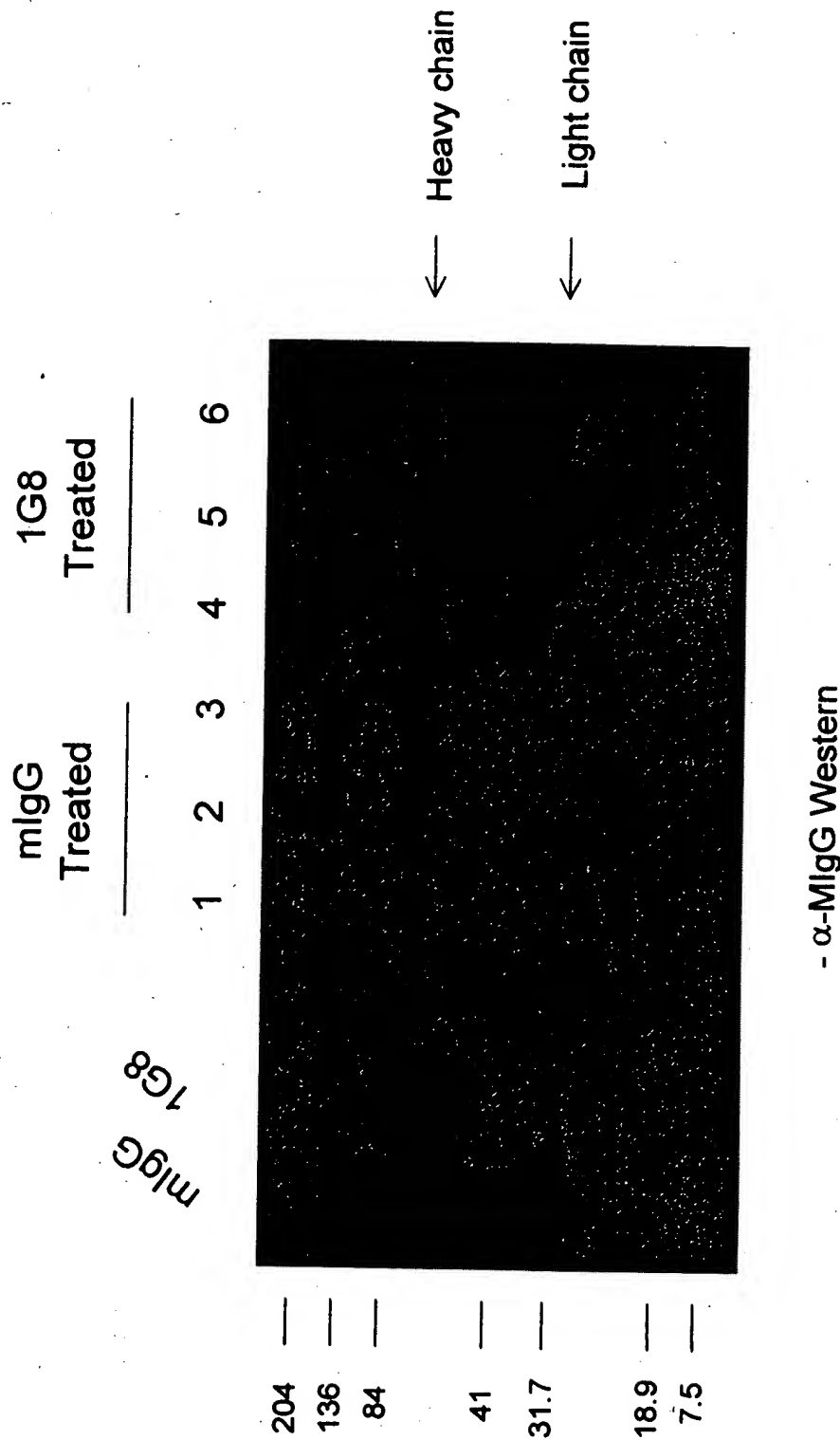
Figure 71

# 3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors



Western blot developed with  $\alpha$ -mIgG/k

# SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAB TO ESTABLISHED LAPC-9 TUMORS



**M thod:** Mice bearing established LAPC-9 tumors (>100 mm<sup>3</sup>) were injected with either mlgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.